International Commission on Microbiological Specifications for Foods (ICMSF)

Microorganisms in Foods 7

Microbiological Testing in Food Safety Management

Second Edition

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International Commission on Microbiological Specifications for Foods (ICMSF)
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Introduction to the Second Edition

Since the release of the first edition of *Microorganisms in Food 7: Microbiological Testing in Food Safety Management*, the role of microbiological testing in food safety risk management has continued to evolve at a rapid rate. This reflects advances in microbiological testing methods and continuing expansion of the role of risk analysis concepts and techniques in food safety programs and policies. This includes the International Commission on Microbiological Specifications for Foods (ICMSF) release of *Microorganisms in Food 8: Use of Data for Assessing Process Control and Product Acceptance*, which included examples of how a risk-based approach can be used to implement risk-based microbiological testing programs as part of a food safety risk management program.

As a result of the evolving landscape in microbiological food safety, the ICMSF has updated and expanded *Microorganisms in Food 7: Microbiological Testing in Food Safety Management* to communicate new knowledge, its current thinking, and examples of the role of microbiological testing within a risk analysis framework. As before, the second edition was developed by the commission and a limited number of consultants.

In addition to the goals and topics covered in the first edition (see Preface/Introduction to the First Edition), the ICMSF has substantially enhanced the second edition by augmenting the chapters covered in the first edition and adding several new chapters. This includes several new examples of the application of risk-based testing that have built upon the development of a quantitative or semiquantitative risk assessment, the risk-based development of mitigation strategies, and the development of risk-based microbiological sampling plans. The second edition includes new or updated examples related to:

- Aflatoxin in peanuts
- *Salmonella* and *Cronobacter* in powdered infant formula
- *Listeria monocytogenes* in ready-to-eat deli meats
- Enterohemorrhagic *Escherichia coli* on green leafy vegetables
- Viruses in oysters
- *Campylobacter* spp. in chicken meat

Each of the "example chapters" includes a risk evaluation (hazard identification, hazard characterization, exposure evaluation, and risk characterization), a risk management option evaluation (potential control measures; establishment of an Appropriate Level of Protection/Food Safety Objective (ALOP/FSO); performance, process, and product criteria; acceptance criteria for the final product), and consideration of Good Hygienic Practice (GHP), Hazard Analysis and Critical Control Point (HACCP), and regulatory criteria.

Several new or substantially augmented chapters have also been added to the second edition. These include:

- Sampling, Sample Handling, Sample Analysis, and Laboratory Quality Assurance
- Impact of Sampling Concepts on the Effectiveness of Microbiological Methods
- Sampling to Access Control of the Environment
- Tightened, Reduced and Investigational Sampling

As before, the second edition has been written with the dual goal of advancing risk-based food safety concepts with practical guidance to maximize the effectiveness of microbiological sampling programs while being cognizant of their limitations. The book has been written to take into account the needs of a broad readership including the food industry, national and international government agencies, as well as the academic and research communities. As always, the ICMSF would greatly appreciate comments and suggestions on how the commission can better meet our goal of enhancing a broad understanding of the role of microbiological testing in food safety risk management.

Introduction to the First Edition

Microorganisms in Foods 7: The Role of Microbiological Testing in Systems Managing Food Safety (2001) was written by the ICMSF with assistance from a limited number of consultants.

Microorganisms in Foods 7 is based upon Part I of *Microorganisms in Foods 2: Sampling for Microbiological Analysis: Principles and Specific Applications (2nd ed. 1986).* In the 1980s, the control of food safety was largely by inspection and compliance with hygiene regulations, together with end product testing. *Microorganisms in Foods 2* put such testing on a sounder statistical basis through sampling plans, which remain useful at port of entry when there is no information on the conditions under which a food has been produced or processed. At an early stage, the commission recognized that no sampling plan can ensure the absence of a pathogen in food. Testing foods at ports of entry, or elsewhere in the food chain, cannot guarantee food safety.

This led the commission to explore the potential value of HACCP for enhancing food safety, particularly in developing countries. *Microorganisms in Foods 4: Application of the Hazard Analysis Critical Control Point (HACCP) System to Ensure Microbiological Safety and Quality (1988)* illustrated the procedures used to identify the microbiological hazards in a practice or a process, to identify the critical control points at which those hazards could be controlled, and to establish systems by which the effectiveness of control could be monitored. Recommendations are given for the application of HACCP from production/harvest to consumption, together with examples of how HACCP can be applied at each step in the food chain.

Effective implementation of HACCP requires knowledge of the hazardous microorganisms and their response to conditions in foods (e.g., pH, a_w, temperature, preservatives). The commission concluded that such information was not collected together in a form that could be assessed easily by food industry personnel in quality assurance, technical support, and research and development and by those in food inspection at local, state, regional, or national levels. *Microorganisms in Foods 5: Characteristics of Microbial Pathogens (1996)* is a thorough, but concise, review of the literature on growth, survival, and death responses of foodborne pathogens. It is intended as a quick reference manual to assist making judgments on the growth, survival, or death of pathogens in support of HACCP plans and to improve food safety.

Microorganisms in Foods 6: Microbial Ecology of Food Commodities (1998) is intended for those primarily in applied aspects of food microbiology such as food processors, food microbiologists, food technologists, veterinarians, public health workers, and regulatory officials. For 16 commodity areas, it describes the initial microbial flora, the prevalence of pathogens, the microbiological consequences of processing, typical spoilage patterns, episodes implicating those commodities with foodborne illness, and measures to control pathogens and limit spoilage.

This book, *Microorganisms in Foods 7: The Role of Microbiological Testing in Systems Managing Food Safety (2001)*, illustrates how systems such as HACCP and GHP provide greater assurance of

safety than microbiological testing but also identifies circumstances where microbiological testing still plays a useful role in systems to manage food safety. It continues to address the commission's objectives to (a) assemble, correlate, and evaluate evidence about the microbiological safety and quality of foods; (b) consider whether microbiological criteria would improve and ensure the microbiological safety of particular foods; (c) propose, where appropriate, such criteria; (d) recommend methods of sampling and examination; and (e) give guidance on appraising and controlling the microbiological safety of foods.

This book introduces the reader to a structured approach for managing food safety, including sampling and microbiological testing. The text outlines how to meet specific food safety goals for a food or process using Good Hygienic Practice (GHP) and the HACCP system.

The concept of a Food Safety Objective (FSO) is recommended to industry and control authorities to translate "risk" into a definable goal for establishing food safety management systems that incorporate the principles of GHP and HACCP. FSOs provide the scientific basis for the industry to select and implement measures that control the hazard(s) of concern in specific foods or food operations, control authorities to develop and implement inspection procedures to assess the adequacy of control measures adopted by the industry, and quantify the equivalence of inspection procedures in different countries.

Microbiological testing can be a useful tool in the management of food safety. However, microbiological tests should be selected and applied with knowledge of their limitations, as well as their benefits and the purposes for which they are used. In many instances, other means of assessment are quicker and more effective.

The need for microbiological testing varies along the food chain. Points in the food chain where information about the microbiological status of a food will prove most useful for control purposes should be selected. Similarly, in a food operation, samples may be collected from different points in a process for control purposes.

Finally, a framework is provided by which importing countries can assess whether foods from other countries have been produced in a manner that provides a level of protection equivalent to that required for domestically produced foods.

This book illustrates the insensitivity of even statistically based sampling plans and encourages a rational approach to the use of microbiological testing in systems that manage food safety through GHP and HACCP. Several new chapters are based on the experience of the food industry in controlling salmonellae, *Listeria monocytogenes*, and *Escherichia coli* O157:H7, on tightened or investigational sampling, on microbiological testing of the processing environment, and on the use of statistical process control to detect trends and work toward continuous improvement.

The book is intended to be useful for anyone who is engaged in setting microbiological criteria, be it for the purpose of governmental food inspection and control or the industry. For students in food science and technology, it offers a wealth of information on food safety management and many references for further study.

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Contents

1 Microbiological Hazards and Their Control 1
1.1 Introduction 1
1.2 Historical Perspective on Introducing Risk-Based Metrics 3
1.3 The Concepts of a Food Safety Management System 6
1.4 Historical Development of Modern Food Safety Management 10
1.5 Foodborne Illness: Etiologic Agents and Contaminants 13
1.5.1 Bacteria 13
1.5.2 Viruses 15
1.5.3 Protozoa 16
1.5.4 Seafood Toxins 16
1.5.5 Toxigenic Fungi 17
1.6 Surveillance and Trends in Foodborne Illness 17
1.7 Importance of Effective Control Measures 18
1.8 Effectiveness of Prerequisite Programs and HACCP 20
1.9 Would PO/FSO Improve Food Safety and Reduce Foodborne Illness? 21
1.10 Use of PO/FSO in Food Safety Management 21
1.11 Performance, Process, Product, and Default Criteria 22
1.12 Assessment of Control of a Process 24
1.13 Acceptance Criteria 24
1.14 Microbiological Testing and Criteria 24
1.15 Summary 25
References 25

2 Evaluating Risks and Establishing Food Safety Objectives and Performance Objectives 31
2.1 Introduction 31
2.2 Managing Tolerable Level of Risk 36
2.3 Importance of Epidemiologic Data 38
2.4 Evaluation of Risk 42
2.4.1 Introduction 42
2.4.2 Use of Expert Panels 44
2.5 Food Safety Objectives and Performance Objectives 45
2.5.1 Examples of FSOs/POs 46
2.5.2 The Use of FSOs and POs 48

2.6 Establishment of an FSO/PO Based on a Risk Evaluation by an Expert Panel 50
2.7 Evaluation of Risk by Detailed Qualitative/Quantitative Risk Assessments 51
2.7.1 Quantitative Risk Assessment 51
2.7.2 Hazard Identification 52
2.7.3 Exposure Assessment 52
2.7.4 Hazard Characterization 53
2.7.5 Risk Characterization 54
2.7.6 Mathematical Approaches 55
2.8 Establishment of an FSO Based on Quantitative Risk Assessment 56
2.9 Stringency of FSOs/POs in Relation to Risk and Other Factors 57
2.10 Summary 57
References 58

3 Meeting FSO and PO Through Control Measures 63
3.1 Introduction 63
3.2 Control Measures 64
3.2.1 Good Hygiene Practices (GHP) 65
3.2.2 Hazard Analysis and Critical Control Point (HACCP) 66
3.3 Confirmation that the FSO/PO Is Technically Achievable 68
3.4 Importance of Control Measures 70
3.4.1 Examples of the Effectiveness of Control Measures 70
3.5 Deriving Performance Criteria 80
3.5.1 Examples of General Use of the ICMSF Conceptual Equation 83
3.5.2 Special Cases Requiring a Refined Approach to the Use of the Equation 90
3.6 Process and Product Criteria 92
3.7 The Use of Microbiological Sampling and Performance Criteria 92
3.8 Quantitative Performance of Attributes Sampling Plans 93
3.9 Default Values 95
3.10 Process Validation 95
3.10.1 Laboratory Challenge Tests 96
3.10.2 Data Collected From Food Operations 97
3.10.3 Process Variability 97
3.11 Monitoring and Verifying Control Measures 99
3.12 Examples of Control Options 99
3.13 Assessing Equivalency of Food Safety Management Systems 101
References 101

4 Selection and Use of Acceptance Criteria 105
4.1 Introduction 105
4.2 Equivalence 106
4.3 Establishment of Acceptance Criteria 107
4.3.1 Types of Acceptance Criteria 107
4.4 Application of Acceptance Criteria 108
4.5 Determining Acceptance by Approval of Suppliers in Business-to-Business Relationships 109
4.5.1 Role of FSO and PO in the Approval of a Supplier 109
4.5.2 Approval Procedures 110
4.6 Factors that May Affect a Decision on Lot Acceptance 112
4.7 Auditing Food Operations for Supplier Acceptance 113
4.7.1 Further Considerations for Auditing a Supplier When a FSO/PO Has Been Established 114
References 115

5 Establishment of Microbiological Criteria . . . 117
5.1 Introduction . . . 117
5.2 Purposes and Application of Microbiological Criteria for Foods . . . 118
5.3 Definition of Microbiological Criterion . . . 119
5.4 Types of Microbiological Criteria . . . 119
5.4.1 Microbiological Standards . . . 119
5.4.2 Microbiological Guidelines . . . 119
5.4.3 Microbiological Specifications . . . 120
5.5 Application of Microbiological Criteria . . . 120
5.5.1 Application by Regulatory Authorities . . . 120
5.5.2 Application by Food Businesses . . . 121
5.6 Principles for the Establishment of Microbiological Criteria . . . 122
5.7 Components of Microbiological Criteria for Foods . . . 123
5.7.1 Microorganisms and Their Toxins/Metabolites of Importance in a Particular Food . . . 123
5.7.2 Microbiological Limits . . . 125
5.7.3 Sampling Plans, Sampling Procedures and Handling of Samples Prior to Analysis . . . 126
5.7.4 Microbiological Methods . . . 127
5.7.5 Reporting . . . 127
5.8 Example of Microbiological Criteria for Egg Products . . . 127
References . . . 128

6 Concepts of Probability and Principles of Sampling . . . 131
6.1 Introduction . . . 131
6.2 Probability . . . 131
6.3 Population and Sample of the Population . . . 132
6.4 Choosing the Sample Units . . . 132
6.5 The Sampling Plan . . . 133
6.6 The Operating Characteristic Function . . . 133
6.6.1 The OC-Curve . . . 134
6.6.2 The Binomial Distribution . . . 135
6.6.3 Consumer Risk and Producer Risk . . . 137
6.6.4 Stringency and Discrimination . . . 138
6.7 Acceptance and Rejection . . . 140
6.8 What Is a Lot? . . . 140
6.9 What Is a Representative Sample? . . . 140
6.10 Confidence in Interpretation of Results . . . 141
6.11 Practical Considerations . . . 142
References . . . 143

7 Sampling Plans . . . 145
7.1 Introduction . . . 145
7.2 Attributes Plans . . . 145
7.2.1 Two-Class Attributes Plans . . . 145
7.2.2 Three-Class Attributes Plans . . . 147
7.3 Variables Plans . . . 152
7.3.1 Identification . . . 153
7.3.2 Prescribing Confidence in Decisions . . . 153
7.3.3 Operation . . . 153

7.4 Comparison of Sampling Plans . . . 155
7.4.1 General Remarks . . . 155
7.4.2 Determining the Concentration of Microorganisms Controlled by Attributes Sampling Plans . . . 156
7.4.3 Comparison of Two-Class and Three-Class Attributes Plans . . . 156
7.4.4 Construction of Three-Class Plans Using Previous Information . . . 160
7.4.5 Comparison of Three-Class Attributes and Variables Plans . . . 162
References . . . 163

8 Selection of Cases and Attributes Plans . . . 165
8.1 Introduction . . . 165
8.2 Microbial Criteria: Utility, Indicator and Pathogenic Microorganisms . . . 166
8.2.1 Utility Microorganisms . . . 166
8.2.2 Indicator Microorganisms . . . 166
8.2.3 Pathogens . . . 167
8.3 Factors Affecting the Risk Associated with Pathogens . . . 167
8.3.1 Epidemiologic Considerations . . . 167
8.3.2 Ecological Features . . . 168
8.3.3 Clinical Features . . . 169
8.3.4 Diagnostic Considerations . . . 170
8.4 Categorizing Microbial Hazards According to Risk . . . 170
8.4.1 Moderate Hazards . . . 170
8.4.2 Serious Hazards, Incapacitating, But Not Life-Threatening . . . 171
8.4.3 Severe Hazards, Life Threatening . . . 171
8.5 Definition of Cases . . . 178
8.5.1 Factors Impacting the Case Choice . . . 179
8.5.2 Choosing Appropriate Cases: Examples . . . 181
8.6 Deciding Between 2-Class and 3-Class Attributes Sampling Plans . . . 182
8.7 Determining Values for m and M . . . 183
8.8 Specific Knowledge About the Lot . . . 184
8.9 What Is a Satisfactory "Probability of Acceptance"? . . . 185
8.10 Selecting n and c . . . 186
8.11 Arithmetic and Geometric Means . . . 187
8.12 Sampling Plan Performance of the Cases . . . 189
8.13 The ICMSF Tool . . . 195
References . . . 195

9 Sampling, Sample Handling, Sample Analysis and Laboratory Quality Assurance . . . 197
9.1 Introduction . . . 197
9.2 Collection of Sample Units . . . 198
9.2.1 Investigative Sampling . . . 199
9.2.2 Containers and Sampling Equipment . . . 199
9.2.3 Sampling Procedures . . . 200
9.2.4 Sample Labelling . . . 202
9.3 Retention Samples . . . 202
9.4 Intermediate Storage and Transportation . . . 203
9.5 Reception of Samples . . . 203
9.6 Sample Analysis . . . 203

9.6.1 Withdrawing Analytical Units . . . 204
9.6.2 Dilution and Homogenization of the Analytical Unit . . . 204
9.6.3 Recovery of Injured Cells . . . 205
9.7 Discrepancies Between Original and Retest Results . . . 206
9.7.1 Non-random Distribution . . . 207
9.7.2 Low Prevalence of Contamination in a Lot or Batch . . . 209
9.7.3 Low Prevalence of Contamination Across Many Batches . . . 210
9.7.4 Change in the Concentration of Contamination . . . 210
9.8 Errors Associated with Methods . . . 211
9.9 Laboratory Quality Assurance . . . 212
9.9.1 Laboratory Accreditation . . . 212
9.9.2 Validation of Methods . . . 213
9.9.3 Good Laboratory Practices and Molecular Techniques . . . 213
9.9.4 Proficiency Testing . . . 214
References . . . 214

10 Impact of Sampling Concepts on the Effectiveness of Microbiological Methodologies . . . 219
10.1 Introduction . . . 219
10.2 Impact of Microbial Injury on the Effectiveness of Sample Plans . . . 220
10.3 Impact of Enrichment Efficiency on the Effectiveness of Sampling Plans . . . 221
10.3.1 Effect of Extent of Growth in Enrichment Broth . . . 222
10.3.2 Effect of the Volume of Broth from Completed Enrichment Examined for Target Microorganism . . . 223
10.3.3 Effects of Incubation Time and Target Microorganism Growth Rate . . . 224
10.4 Impact of Microbial Competition and Mixed Microbiota on Effectiveness of Detection Methods and Ultimately Sampling Plans . . . 227
10.4.1 The Potential Impact of the Jameson Effect on the Effectiveness of Sampling Plans . . . 229
10.4.2 Effect of Differential Growth Rates on the Confirmation of the Identity of Pathogenic Species or Strains . . . 230
10.5 Impact of Immunomagnetic Concentration Systems on the Effectiveness of Sampling Plans . . . 232
10.6 Determination of Sample Size in Non-cultural Microbiological Methods and Its Impact on the Effectiveness of Sampling Plans . . . 234
10.7 Effect of Specificity and Sensitivity on Sampling Plans and the Operation Characteristic (OC) Curve . . . 235
10.8 Summary . . . 235
References . . . 236

11 Tightened, Reduced and Investigational Sampling . . . 239
11.1 Introduction . . . 239
11.2 Application of Tightened Sampling and Investigational Sampling . . . 243
11.3 Tightened Sampling Plans . . . 245
11.4 Example of the Influence of Sampling Plan Stringency in Detecting and Identifying Defective Lots, and Re-establishing U nder Control Conditions . . . 249
11.5 Selecting the Sampling Plan According to Purpose . . . 250
11.6 Reduced Sampling . . . 251
11.6.1 Skip-Lot Sampling . . . 251
11.7 Considerations in the Implementation of Stringent Sampling Plans . . . 254

11.7.1 Conceivable Alternative Plans . . . 254
11.7.2 Erroneous Procedures for Stringent Sampling Plans . . . 255
11.8 Examples . . . 255
11.8.1 Examples of the Moving Window . . . 255
11.8.2 An Example of Reduced Sampling: Sampling in Slaughterhouses and at Premises Producing Minced Meat and Meat Preparations in Europe . . . 257
11.8.3 An Example of Tightened Sampling: Aseptically-Packed Products . . . 258
11.8.4 Example of Reduced Sampling: Skip-Lot Sampling . . . 260
References . . . 262

12 Sampling to Assess Control of the Environment . . . 263
12.1 Introduction . . . 263
12.2 Importance of Prerequisite Programs. . . . 264
12.3 Microbial Contamination from the Environment. . . . 265
12.3.1 Contamination During Primary Production . . . 265
12.3.2 Environmental Contamination in Processing Facilities . . . 266
12.3.3 Contamination During Distribution, Retail and at the Consumer Level . . . 268
12.4 Establishment and Growth of Foodborne Pathogens in the Processing Environment . . . 269
12.4.1 Transient Versus Established Microorganisms . . . 269
12.5 Measures to Control Pathogens in the Food-Processing Environment . . . 273
12.5.1 Minimizing Entry of Pathogens . . . 273
12.5.2 Minimizing the Establishment of Pathogens in the Environment . . . 274
12.6 Sampling the Processing Environment . . . 275
12.6.1 Assessing the Risk of Product Contamination (Investigational Phase) . . . 275
12.6.2 Establishing a Routine Environmental Sampling Program . . . 276
12.6.3 Sampling Locations: The Zone Concept . . . 276
12.6.4 Number, Frequency and Time of Sampling . . . 278
12.6.5 Sample Collection . . . 280
12.6.6 Sample Analysis . . . 280
12.6.7 Managing the Data from Environmental Sampling . . . 281
12.6.8 Investigational Sampling to Determine the Source of Contamination . . . 281
References . . . 284

13 Statistical Process Control . . . 293
13.1 Introduction . . . 293
13.2 Statistical Process Control: An Introduction . . . 296
13.3 Knowledge of the Degree of Variability and the Factors that Influence Variability . . . 297
13.3.1 Establishing a Baseline for a Process . . . 297
13.3.2 Types of Microbiological Data for Baseline. . . . 298
13.3.3 Determining the Causes of Variability . . . 299
13.3.4 Variability in Foods and Food Processing Parameters . . . 299
13.4 Process Capability Study . . . 300
13.4.1 Establishing Criteria and Monitoring Procedures . . . 301
13.4.2 Types of Variability and Error . . . 302
13.5 Monitoring and Verifying a Single Lot of Food: Using SPC during Production. . . . 303

13.6 Organizing Data from Across Multiple Lots of Food to Maintain or Improve Control During Production . . . 304
13.6.1 Variables Charts of Quantitative Data . . . 306
13.6.2 General Principles of Variable Control Chart Construction . . . 307
13.6.3 Corrections When Data Are Auto-Correlated . . . 310
13.6.4 Special Considerations for Charting Individual Counts of Microbial Population Density . . . 311
13.6.5 Selecting Limits for Variable Control Charts of Microbial Counts . . . 311
13.6.6 Caution in Interpreting Certain Types of Variables Charts . . . 313
13.6.7 Other Control Charts that Can Be Used for Microbiological Data . . . 314
13.6.8 Attribute Charts of Presence/Absence Determinations . . . 315
13.6.9 Other Examples of Using Attribute Control Charts . . . 317
13.6.10 Advantages of Multiple Charting of Data Subsets . . . 323
13.6.11 Caution Regarding Control Chart Software . . . 324
13.7 Use of Process Control Testing as a Regulatory Tool . . . 324
13.8 Investigating and Learning from Previously Unrecognized Factors or Unforeseen Events . . . 325
13.9 Conclusions . . . 325
References . . . 327

14 Aflatoxins in Peanuts . . . 329
14.1 Introduction . . . 329
14.2 Risk Evaluation . . . 330
14.2.1 Risk Assessment . . . 330
14.2.2 Exposure Assessment . . . 331
14.2.3 Factors Affecting Exposure Assessment . . . 332
14.2.4 Risk Characterization . . . 333
14.3 Risk Management . . . 333
14.3.1 Principal Control Measures . . . 334
14.3.2 Establishing ALOP/FSO . . . 336
14.3.3 Identifying and Establishing Performance Objectives . . . 336
14.3.4 Performance Criteria . . . 338
14.3.5 Acceptance Criteria . . . 339
14.4 Relating Risk Management Metrics to Stringency of Food Safety System . . . 340
14.4.1 HACCP Plans . . . 340
14.4.2 Good Hygienic Practice (GHP) Programs . . . 340
References . . . 341

15 Pathogens and Indicator Organisms in Powdered Infant Formula . . . 345
15.1 Introduction . . . 345
15.2 Risk Assessment . . . 346
15.2.1 Hazard Identification . . . 346
15.2.2 Exposure Assessment . . . 347
15.2.3 Hazard Characterization . . . 347
15.2.4 Risk Characterization . . . 348
15.3 Risk Management . . . 348
15.3.1 Control Measures . . . 348
15.3.2 ALOP/FSO . . . 351
15.3.3 Identifying and Establishing Performance Objectives . . . 351
15.3.4 Performance Criterion . . . 352
15.3.5 Acceptance Criteria for Final Product . . . 352

15.4 Relating Risk Metrics to Stringency of Food Safety System. 353
15.4.1 HACCP Programs. 353
15.4.2 GHP Programs 353
15.4.3 Regulatory Requirements and Criteria 353
References. 353

16 *Listeria monocytogenes* in Ready-to-Eat Deli-Meats 357
16.1 Introduction 357
16.2 Risk Evaluation 358
16.2.1 Published Risk Assessments/Risk Profiles 358
16.2.2 Exposure Assessment 360
16.2.3 Factors Affecting Dose-Response. 362
16.2.4 Risk Characterization 363
16.3 Risk Management 364
16.3.1 Principal Control Measures 364
16.3.2 Establishing an ALOP/Food Safety Objective 368
16.3.3 Identifying and Establishing Performance Objectives 369
16.3.4 Performance Criteria 374
16.3.5 Acceptance Criteria for Final Product 376
16.4 Relating Risk Management Metrics to the Stringency of a Food Safety System. 377
16.4.1 HACCP Programs. 377
16.4.2 GHP Programs 378
16.4.3 Regulatory Requirements and Criteria 378
References. 379

17 Enterohemorrhagic *Escherichia coli* on Fresh-Cut Leafy Vegetables 385
17.1 Introduction 385
17.2 Risk Evaluation 386
17.2.1 Published Risk Assessments/Risk Profiles 386
17.2.2 Hazard Identification 387
17.2.3 Hazard Characterization 388
17.2.4 Dose Response Considerations. 388
17.2.5 Exposure Assessment 390
17.2.6 Risk Characterization 393
17.3 Risk Management 393
17.3.1 Principal Control Measures 393
17.3.2 Establishing an ALOP/FSO 394
17.3.3 Identifying and Establishing Performance Objectives 395
17.3.4 Performance Criteria 397
17.3.5 Acceptance Criteria 398
17.4 Risk Characterization and Risk Management 400
17.4.1 Deterministic Approaches 400
17.4.2 Exploring Alternative Risk Management Options 400
17.4.3 Stochastic Approaches 403
17.5 Relating Risk Management Metrics to Stringency of Food Safety Systems 406
17.5.1 HACCP Plans 406
17.5.2 GHP Programs 406
17.5.3 GAPs. 406
17.5.4 Regulatory Requirements and Criteria 406
References. 407

18 Viruses in Oysters . . . 411
18.1 Introduction . . . 411
18.2 Risk Evaluation . . . 412
18.2.1 Hazard Identification/Risk Profiles . . . 412
18.2.2 Factors Affecting Hazard Characterization . . . 413
18.2.3 Factors Affecting Exposure Assessment . . . 415
18.2.4 Risk Characterization . . . 418
18.3 Risk Management . . . 419
18.3.1 Pre-harvest Control Measures . . . 419
18.3.2 Post-harvest Control Measures . . . 421
18.3.3 Vaccination Control of HAV . . . 423
18.3.4 Education, Risk Communication . . . 423
18.3.5 Acceptable Levels of Consumer Protection/Establishing ALOP/FSO/PO . . . 423
18.3.6 Identifying and Establishing Performance Objectives/Performance Criteria . . . 424
18.3.7 Acceptance Criteria . . . 426
18.4 Relating Risk Management Metrics to Stringency of Food Safety System . . . 427
18.4.1 HACCP Plans . . . 427
18.4.2 GHP Programs . . . 427
18.4.3 Regulatory Requirements and Criteria . . . 427
References . . . 427

19 *Campylobacter* in Chicken Meat . . . 435
19.1 Introduction . . . 435
19.2 Risk Evaluation . . . 436
19.2.1 Hazard Identification . . . 436
19.2.2 Hazard Characterization . . . 436
19.2.3 Exposure Assessment . . . 438
19.2.4 Risk Characterization . . . 442
19.3 Risk Management . . . 443
19.3.1 Acceptable Level of Consumer Protection . . . 443
19.3.2 Establishing a Food Safety Objective . . . 443
19.3.3 Quantifying a Public Health Goal . . . 444
19.3.4 Control Measures to Achieve a Public Health Goal . . . 445
19.3.5 Establishing Performance Objectives Throughout the Chicken Production Chain to Account for Control Measures . . . 445
References . . . 453

Appendix A: Glossary . . . 457

Appendix B: ICMSF Publications . . . 461

Appendix C: Meetings Sponsors 2010 to 2016 . . . 465

Index . . . 467

Chapter 1
Microbiological Hazards and Their Control

1.1 Introduction

The purpose of this book is to introduce the reader to a structured approach for managing food safety, including setting management goals for food safety management systems, sampling and microbiological testing. This structured approach connects governmental food safety management in the context of public health protection with operational management activities at individual stages of or across a food supply chain. An important feature of the structured approach is that it is based on an appreciation of *risk* rather than the potential presence of a hazard. From the perspective of national competent authorities, the framework of risk analysis (RA) provides a systematic approach to food safety management decision making. Operationally, general food safety management relies heavily on prerequisite programs, such as Good Hygienic Practice (GHP), Good Agricultural Practices (GAP), and Good Manufacturing Practices (GMP). Prerequisite programs support systems based on Hazard Analysis Critical Control Point (HACCP) principles, which manage food safety more specifically and tailored to a particular food business operation. For the purposes of brevity, "prerequisite programs" is used as a collective term throughout this chapter where the suite of good practice programs is relevant.

The International Commission on Microbiological Specifications for Foods (ICMSF or the Commission) recommended that industry[1] and control authorities adopt new risk management metrics referred to as the Food Safety Objective (FSO), Performance Objective (PO), and Performance Criterion (PC) (ICMSF 1998a, 2002, 2010, 2011). Jointly, these metrics were established to provide a bridge between traditional food safety metrics (i.e., microbiological criteria, process criteria and product criteria) and the concept of Appropriate Level of Protection (ALOP) envisioned in the Sanitary and Phytosanitary (SPS) agreement of the World Trade Organization (WTO 1995), and have been adopted by Codex Alimentarius Commission (CAC 2007a, 2016). Figure 1.1 illustrates how the various metrics link together conceptually. Through the use of FSO and/or PO, an appreciation of the food safety risk at population level (i.e., the ALOP) is translated into definable outcome-oriented food safety management goals at particular steps (or points) in the farm-to-fork continuum. These steps are for instance primary production, manufacturing, distribution and sales, or food service. To meet the outcome or goal for a step (either the FSO when it concerns the last step in the continuum or otherwise

[1] The term "industry" will mean: an organization, company or group of individuals (cooks) working professionally in the food supply chain from primary agricultural production to the sale to, or preparation of food for, the consumer. The particular meaning in the text will depend on the context in which it is used.

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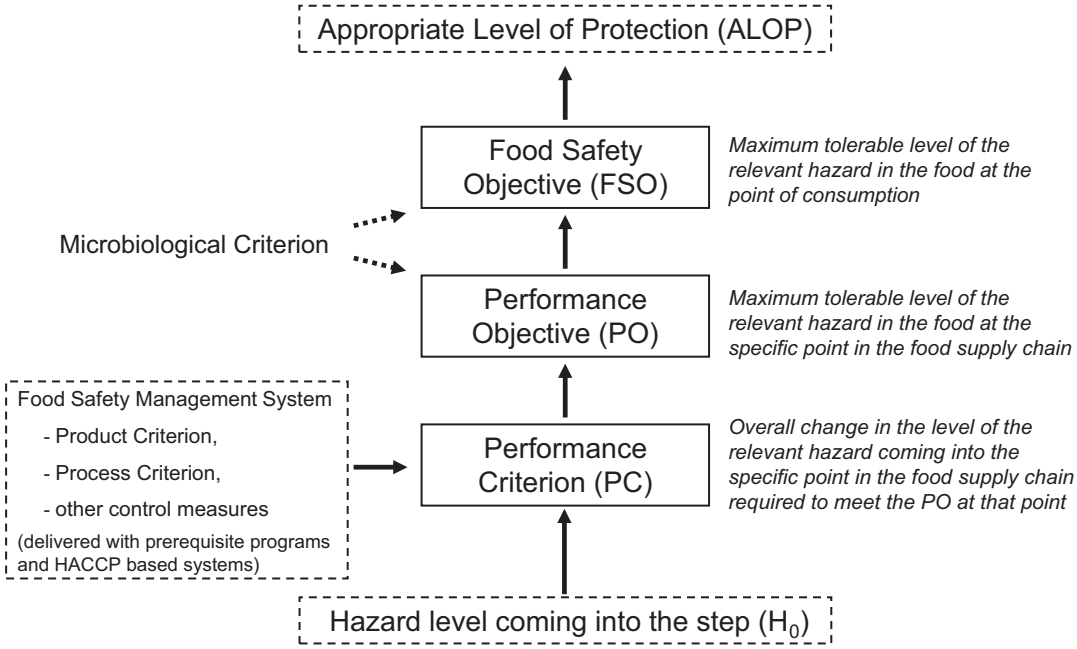
Appropriate Level of Protection (ALOP)
Food Safety Objective (FSO)
Maximum tolerable level of the relevant hazard in the food at the point of consumption
Microbiological Criterion
Performance Objective (PO)
Maximum tolerable level of the relevant hazard in the food at the specific point in the food supply chain
Food Safety Management System
- Product Criterion,
- Process Criterion,
- other control measures
(delivered with prerequisite programs and HACCP based systems)
Performance Criterion (PC)
Overall change in the level of the relevant hazard coming into the specific point in the food supply chain required to meet the PO at that point
Hazard level coming into the step (H_0)

Fig. 1.1 New risk management metrics (FSO, PO and PC) and their use in establishing appropriate food safety management systems aligned with public health goals (e.g. ALOP) and how, in principle, microbiological criteria can be used to verify that FSO/PO levels are met in the food supply chain

the PO), the level of the hazard coming into the step needs to be changed in a controlled fashion and this change is called the PC. Overall, the set of control measures able to deliver the PC for a particular step is referred to as the "food safety management system" for that step and is implemented using prerequisite programs and HACCP approaches. Such control measures may relate to the pH or water activity (a_w) of a food governed by so-called product criteria; they may also relate to a particular treatment to be applied such as pasteurization, for which the governing parameters are referred to as process criteria. A microbiological criterion may be set on the basis of a PO or an FSO (the latter is generally not practical) in instances where testing of foods for a specific microorganism can be an effective means to verify that these goals are met.

In this book, the term food safety management system is used broadly to include single or multiple control measures used in the production, manufacture, and distribution up to final preparation of a food or food components at any stage of the food chain. Codex uses an equivalent term "food safety control systems" (CAC 2008a). The ability to articulate the expected performance of a food safety management system in terms of the necessary management of public health risks is a critical component of the RA paradigm evolving in Codex Alimentarius (CAC 2007a). Within this paradigm, FSOs/POs provide the scientific basis for industry to select and implement measures that control the hazard(s) of concern in specific foods or food operations, for control authorities to develop and implement inspection procedures to assess the adequacy of control measures adopted by industry, and for quantifying the equivalence of inspection procedures in different countries. Microbiological testing can be a useful tool in the management of food safety. However, microbiological tests should be selected and applied with knowledge of their limitations, as well as their benefits and the purposes for which they are used. In many instances, other means of assessment are quicker and equally or even more effective.

The need for microbiological testing varies along the food supply chain. Points should be selected in the food supply chain where information about the microbiological status of a food will prove most useful for control purposes. Similarly, in a food operation, samples may be collected from different points in a process for control purposes.

A framework is provided by which importing countries can assess whether foods from other countries have been produced in a manner that provides a level of protection equivalent to that required for domestically produced foods.

Guidance is provided for establishing sampling plans based on risk to consumers. In Chap. 8, fifteen "cases" are described that take into account whether the hazard will increase, decrease, or not change between the point where a food is sampled and the point that food is consumed. These same principles are useful for foods at port-of-entry and in domestic situations when the safety and acceptability of a food is uncertain.

1.2 Historical Perspective on Introducing Risk-Based Metrics

The ICMSF was asked in 1995 by the Codex Committee on Food Hygiene to write a discussion paper on *The Management of Pathogens in Foods in International Trade*. During its meeting in 1996, the Commission concluded that such management should use existing Codex Alimentarius documents and should be in line with the requirements of the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (WTO/SPS agreement) (WTO 1995), which stated that foods could freely be imported if they would not endanger a country's ALOP concerning consumer health protection. In the same agreement, risk assessment was identified as the tool to determine whether a food would or would not endanger the ALOP. How an ALOP needed to be expressed or how it should be established was not elaborated in the agreement.

At that time a framework for microbiological risk assessment was started by the Codex Commission Committee for Food Hygiene (CAC 1996). Other documents in the Codex system included the General Principles of Food Hygiene, with its annex on HACCP, and *the Principles for the Establishment and Application of Microbiological Criteria for Foods*. The ICMSF recognized that it would be difficult for the food industry to prove that a product would meet the ALOP of an importing country if that ALOP was expressed in terms such as "the number of illnesses per 100,000 of a population caused by a hazard/food combination." This would be an expression of the resulting risk in a consumer population, rather than the consequence of achieving a certain level of control over a hazard by an industry. Moreover, the Codex tools to assure the safety of the food were prerequisite programs and HACCP approaches; it was not evident how these could be best related to the ALOP of an importing country.

In a HACCP-based system, hazards are controlled by their elimination from the food or reduction to acceptable levels defined for a food/hazard combination. It was the understanding of the Commission that these acceptable levels would actually form the basis for a particular ALOP. In other words, an ALOP or the contribution to it related to a particular food/hazard combination would not be surpassed when the relevant hazard in the commodity would be managed to the defined acceptable level. However, as long as an ALOP was not expressed as "the level of a hazard in a food that would be acceptable" the industry would not know what the acceptable level would be to achieve through the use of prerequisite programs and HACCP systems. The ICMSF thus felt the need to develop the concept of an FSO that would be link the ALOP set at the country level to the operational level that industry works at by specifically providing for the required expression of "the level of a hazard in a food that would be acceptable". The FSO concept followed the concept of *quality objectives* in quality assurance and quality management standards (Jouve 1992). Early communications introduced *food safety objectives* in broad terms (Jouve 1996).The same wording had been proposed at congresses at the time to express some form of justification for sanitary measures with respect to determining equivalence, which later also was elaborated on in refereed publications (Hathaway 1997; Hathaway and Cook 1997).

In introducing the concept of FSO, the intention of ICMSF was that the FSO would convert the ALOP or acceptable (tolerable) level of risk (number of cases of illnesses) into the maximum frequency and/or concentration of a hazard considered to be tolerable for consumer protection. That FSO could then be translated into the performance of a food process that would assure that, at the point of consumption, the level of the hazard in a food would not be greater than the value established in the FSO. Risk assessment was considered to be helpful in establishing the FSO, because the risk characterization could be expressed for a country as "the estimated number of illnesses per year", or other terms similar to the ALOP that reflect a country's public health goals for foodborne risks. Information from hazard characterization such as dose-response relationships is key to deriving an FSO from the ALOP, while exposure assessment details could help identify control measures that would assure that the FSO would be met.

Consequently, in 1996, the ICMSF recommended to the Codex secretariat that a step-wise procedure should be used to manage pathogens in foods. The first step would be to perform a microbiological risk assessment; the second step would be to develop an FSO. Step three should confirm that the FSO would be technically achievable by the application of prerequisite programs and HACCP. Step four was the establishment of microbiological criteria, when there was a need, and step five was establishing other acceptance criteria for foods in international trade.

In 1997, an additional step was introduced between steps one and two, i.e., the use of the newly developed concept of risk management. Through the introduction of this step, it was recognized that the establishment of an FSO was not only a scientific risk assessment exercise, but also a societal decision in which the various stakeholders (regulators, academics, consumers, industries, etc.) should participate (ICMSF 1998a). At the same time that the FSO concept was brought into discussion in the Codex Committee on Food Hygiene, the term *food safety objective* was also introduced in the Codex Committee on Import and Export, where it was meant to deal with the outcome of all kinds of sanitary measures. This situation led to confusion about the nature of FSOs, why they are needed, what they should accomplish, etc. The Codex Alimentarius Commission decided in 2000 that the term FSO would no longer be used in the broader sense, but would only be used by the Codex Committee on Food Hygiene for its purposes.

Working on a revision of ICMSF Book 2 (ICMSF 1986), the Commission decided to introduce the FSO concept as the basis for establishing a food safety management system, noting that FSOs should be broader than just converting an ALOP into a level of a hazard in a food. It was also recognized that a full risk assessment, according to the Codex procedures, would in many cases not be necessary to determine from a governmental perspective what control measures could be required to meet an FSO, but that an expert panel and a less detailed risk evaluation could suffice in many cases. It was clear though that, operationally, prerequisite programs and HACCP would be the systems with which to implement adequate product and process designs that ensure meeting an FSO for a particular hazard, additionally to controlling all other relevant hazards for the particular operation, and these would be established following Codex guidelines.

Originally, the FSO concept was linked to the ALOP concept as described in the WTO/SPS agreement (WTO 1995), which would restrict the ALOP to express an acceptable level of health impact in terms of what is achieved in a particular country at the time that the ALOP is defined. It was later recognized that Codex Alimentarius not only had the task of producing procedures and guidelines that could be used by WTO, but that it also should help nations to improve food safety. In this light, FSOs could not only be seen as levels of hazards that were already achieved in a country (i.e. as a "status quo" benchmark), but also as levels that could be achieved as part of a country's food safety enhancement programs as later discussed for instance in FAO/WHO (2006a). This situation has led to some confusion. On the one hand, a country cannot ask of an exporting country that the exported food meets higher requirements (i.e. more stringent FSOs) than are achieved currently by the industry in the importing country. On the other hand, FSOs used in a food safety enhancement program within a country may be regarded separately from FSOs used to manage foods in international trade. The ICMSF recognized this situation in the 1st edition of ICMSF Book 7 (ICMSF 2002), recommending that FSOs can be used for both purposes, and that governments establish them to communicate to industries the maximum level of a hazard in a food that must not be surpassed. While a risk assessment was considered to be

helpful in establishing the FSO, as discussed above, the Commission recommended that FSOs should also consider available epidemiological evidence that a certain level of a hazard in a food does not cause an intolerable public health problem. If evidence exists that a certain level of a hazard in a food is indeed unacceptable, lower levels should be set if they can be obtained by control measures that are technically achievable with acceptable costs. How FSOs should be established for the various purposes might vary from situation to situation and general guidelines were given (ICMSF 1998a, 2002).

Subsequent discussions within Codex Alimentarius as well as elsewhere (ILSI-Europe 2004; ICMSF 2004; Anonymous 2005; FAO/WHO 2006a) led to the establishment of a set of three new risk-based metrics instead of the original FSO concept proposed by the ICMSF, which had apparently been quite unclear for many stakeholders regarding its purpose, establishment and use. An important step to internationally establishing risk-based metrics was taken by the Codex Committee for Food Hygiene when it proposed these new metrics with specific accompanying definitions, that were accepted by the Codex Alimentarius Commission and included in the procedural manual under the definitions of risk analysis terms related to food safety (CAC 2016) and in the Principles and Guidelines for the Conduct of Microbiological Risk Management (CAC 2007a). Table 1.1 provides definitions for a range of terms used in risk-based food safety management as agreed on by Codex Alimentarius, which includes the definitions of these three new risk-based metrics. More recently, the commission published documents that relate traditional metrics such as microbiological criteria to food safety objectives (van Schothorst et al. 2009) and the validation of control measures using the FSO concept (Zwietering et al. 2010).

Table 1.1 Definitions of risk analysis terms related to food safety (CAC 2016)

Hazard	A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect
Risk	A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food
Risk Analysis	A process consisting of three components: risk assessment, risk management and risk communication
Risk Profile	The description of the food safety problem and its context
Risk Assessment	A scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization
Risk Management:	The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options
Risk Communication	The interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions
Hazard Identification	The identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods
Hazard Characterization	The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents which may be present in food. For chemical agents, a dose-response assessment should be performed. For biological or physical agents, a dose-response assessment should be performed if the data are obtainable
Exposure Assessment	The qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant
Risk Estimate	The quantitative estimation of risk resulting from risk characterization
Food Safety Objective (FSO)	The maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP)
Performance Objective (PO)	The maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption that provides or contributes to an FSO or ALOP, as applicable
Performance Criterion (PC)	The effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a PO or an FSO

1.3 The Concepts of a Food Safety Management System

Chapters 2, 3, 4 and 5 describe in detail a sequence of activities for establishing a comprehensive food safety management system, as summarized in Fig. 1.2. The respective roles of industry and government are described because it is through their collective activities that effective food safety management systems are developed and verified. A series of steps is described for a situation in which an improvement in the control over a hazard in a given food impacts on the food safety systems in place.

Risk managers in government use epidemiologic evidence to link and quantify the level of human illness associated with a microbiological agent and, if possible, a food. They may also develop a view

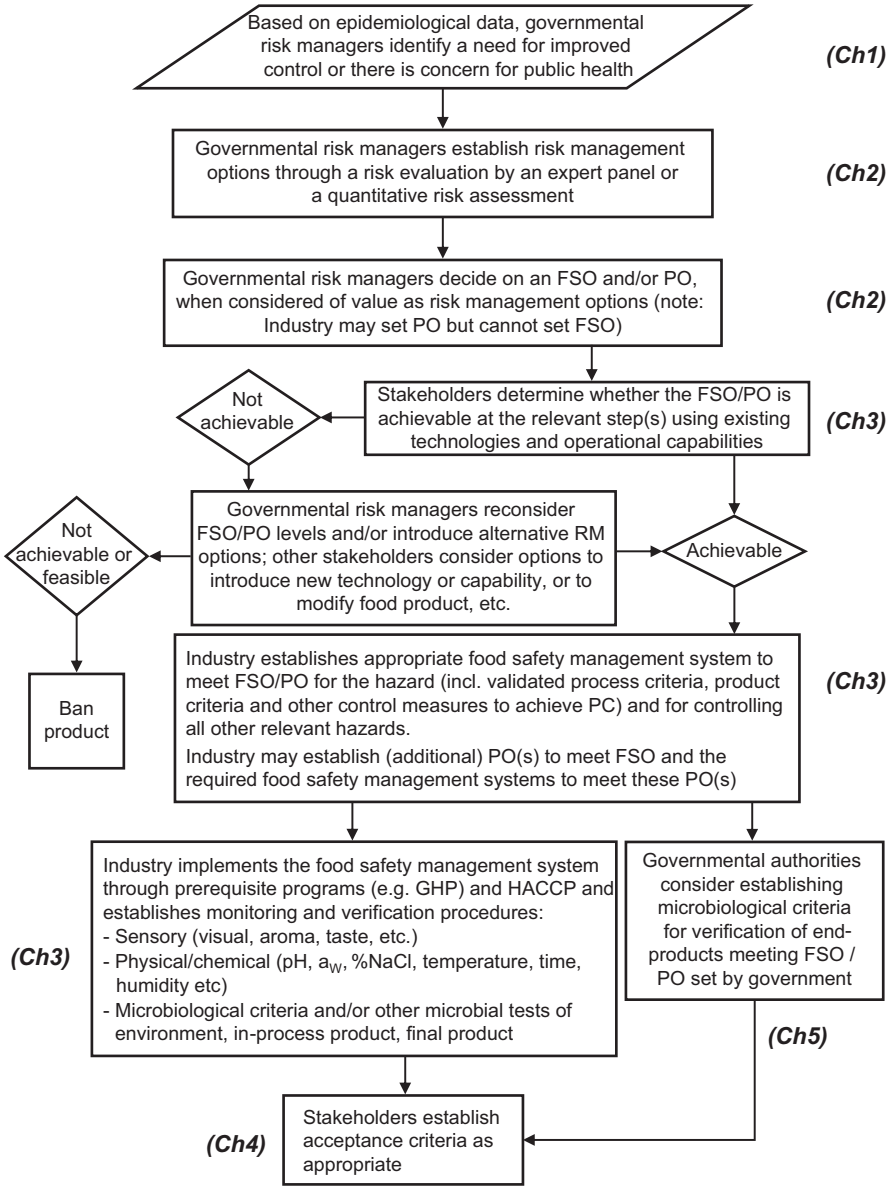
Based on epidemiological data, governmental risk managers identify a need for improved control or there is concern for public health
(Ch1)
Governmental risk managers establish risk management options through a risk evaluation by an expert panel or a quantitative risk assessment
(Ch2)
Governmental risk managers decide on an FSO and/or PO, when considered of value as risk management options (note: Industry may set PO but cannot set FSO)
(Ch2)
Not achievable
Stakeholders determine whether the FSO/PO is achievable at the relevant step(s) using existing technologies and operational capabilities
(Ch3)
Not achievable or feasible
Governmental risk managers reconsider FSO/PO levels and/or introduce alternative RM options; other stakeholders consider options to introduce new technology or capability, or to modify food product, etc.
Achievable
Ban product
Industry establishes appropriate food safety management system to meet FSO/PO for the hazard (incl. validated process criteria, product criteria and other control measures to achieve PC) and for controlling all other relevant hazards.
Industry may establish (additional) PO(s) to meet FSO and the required food safety management systems to meet these PO(s)
(Ch3)
(Ch3)
Industry implements the food safety management system through prerequisite programs (e.g. GHP) and HACCP and establishes monitoring and verification procedures:
- Sensory (visual, aroma, taste, etc.)
- Physical/chemical (pH, a_W, %NaCl, temperature, time, humidity etc)
- Microbiological criteria and/or other microbial tests of environment, in-process product, final product
Governmental authorities consider establishing microbiological criteria for verification of end-products meeting FSO / PO set by government
(Ch5)
(Ch4)
Stakeholders establish acceptance criteria as appropriate

Fig. 1.2 Proposed scheme for establishing a risk-based food safety system to manage the safety of a hazard/food combination (in parentheses the relevant book chapters are indicated)

on the level of an illness in response to a public health concern. Depending on urgency, severity, risk, likelihood of further occurrences, the hazard-food combination, and other factors, risk managers may decide to establish an expert panel to develop recommendations, ask risk assessors to compile a "rough risk assessment" or "risk profile" (often bringing together readily available data) or ask risk assessors to elaborate a more detailed risk assessment (generally requiring data collection and new data generation). Given the food safety issue and the overall situation, these approaches may help the responsible risk manager decide on the best course of action for achieving risk management decisions in a timely fashion. In commissioning an expert elicitation, a risk profile, or a risk assessment, risk managers may consider what new insights and data regarding the hazard/food combination are needed to make an informed decision supported by appropriate scientific and epidemiological data. In any case, a decision is made by the risk manager whether the prevailing burden of illness can be tolerated (which then would fit the description of an ALOP in WTO terms) or whether intervention is required to reduce/ prevent the burden of illness. In the latter case, a new policy for foods being placed on the market locally or through food imports is needed (which would be more in line with an ALOP in the context of a food safety enhancement program). Additional information may be required to better understand the risk management options available for reduction/prevention and the extent to which these options would be feasible in practice. Risk managers could seek to obtain such additional information for instance through further iterations of risk assessment or expert elicitation. Importantly, deciding on an ALOP is a societal decision and the responsibility for the decision needs to be held at the appropriate political level with possible delegation of responsibilities to the relevant competent authority in a country.

Whether a governmental risk manager in a country aims to identify risk management options to prevent an increase in the existing burden of illness or aims to improve the stringency of control of the hazard-food combination such that a certain level of reduction or prevention is achieved, a risk management option to consider is establishing an FSO for the hazard-food combination. The FSO would specify the maximum frequency (also referred to as "prevalence") and/or concentration of the microbiological hazard concerned in the particular food at the time of consumption (CAC 2007a). When this FSO is met by the industry involved in the management of the relevant food supply chain, the governmental risk manager would be confident that the country's ALOP is achieved or contributed to as expected. Conceivably, when meeting a particular FSO achieves a specific ALOP, the hazard/food combination for which the FSO has been set is solely linked to the illness in the population for which the ALOP was established. However, where a given hazard may be associated with quite different foods or food categories and the ALOP covers all of these while different FSOs have been established for individual foods or food categories, meeting an individual FSO may merely contribute to achieving an overall ALOP. The latter case may occur relatively frequently and a good understanding the relative contribution of different hazard/food combinations to the overall ALOP on the basis of epidemiological data, risk assessment or other available information will be important for monitoring and review of the ALOP and associated FSOs.

As noted before, establishing an ALOP is a societal decision in the hand of the relevant competent authority or legislative or regulatory body. Likewise, it is a societal decision whether it is the appropriate course of action for risk managers in government to ensure that the existing burden of illness in a country is not increasing or that further interventions should be put in place for reduction/prevention. Consequently, deriving an FSO from an ALOP equally is a decision informed by societal considerations and thus only a competent authority responsible for food safety would be expected to actually establish FSOs for the country and the food products that they have jurisdiction over.

Having considered setting an FSO as a possible risk management option, governmental risk managers would consult the affected industry and other relevant stakeholders to assess whether the FSO is achievable with current or improved technologies, processes, and practices. It would be prudent to establish through consultation whether achieving an FSO can be monitored and verified, how this may best be done and when a review of the impact of the risk management measure would be appropriate.

Most often, the point of consumption is not a suitable point to monitor and verify whether the foreseen level of control is achieved, since it is impractical from a governmental governance perspective as well as from the industry's food safety assurance perspective. Evidently, it will be difficult or even impossible to generate meaningful monitoring data at the point of consumption, but most importantly at this late stage in the food supply chain the monitoring data generated cannot be made available in a timely manner so as to inform corrective actions.

Based on sufficient understanding of the dynamics of a hazard throughout the course of the food supply chain, a more suitable point for governmental or industry monitoring/verification may be found at earlier steps in the food supply chain than at the point of consumption. Such a point is referred to as the *Performance Objective* (PO) and it specifies the maximum frequency and/or concentration of the hazard in the food concerned at a particular step in the food chain (CAC 2007a). Meeting the PO would mean that the FSO it has been derived from would either be met or would be contributed to as expected. A situation in which a PO would be equal to the FSO is where the hazard level does not change after the step for which the PO has been set up to the point of consumption. This is typically limited to instances where growth of the pathogen is not supported by the conditions of the food or the food environment from the PO point onward. A situation where meeting a PO would contribute to meeting the related FSO could for instance be for a PO that has been set at primary production or at manufacture considering that during the steps following the PO point there may be opportunity for growth of the hazard in the food or for an increase of the hazard level due to cross-contamination; in this example, the food safety management system of steps subsequent to the PO need to be arranged such that the hazard is controlled sufficiently to meet the FSO at the point of consumption. Evidently, multiple POs could be used in such a food supply chain to help monitor and verify whether the required level of control is achieved.

Different from setting an FSO, which is the sole prerogative of governmental risk managers, POs can be set by industry to systematically ensure product safety as a food moves through a food supply chain. Establishing such PO levels at appropriate points in a particular food supply chain, likely involves communication and coordination between different industries sectors or food business operators (FBOs) that control specific steps in that chain. Individual industry sectors or FBOs may derive a PO for one or for several steps under their control. In certain cases, risk managers in government may wish to specify a PO for an industry that may not be able to derive an appropriate PO by their own means, which may be considered as a "safe haven" or "default" risk management option. Appropriate consultation of relevant stakeholders, such as food business operators in the affected industry and others potentially impacted, regarding the feasibility of meeting such a default PO would be best practice.

In order to meet an FSO or a PO, the responsible industry sector(s) or FBO(s) need to establish a suitable food safety management system, based on an adequate product and process design for the end product and considering the intended consumer use (Gorris et al. 2006). The product and process design should rely only on sufficiently validated control measures, which may include process criteria (e.g., pasteurizing at 71 °C for 2.5 min), product criteria (e.g., pH, a_w) and control measures such as physical separation of raw and processed foods to avoid cross-contamination. Risk assessment techniques may be deployed in assessing appropriate control measures (Membre et al. 2007; van Schothorst et al. 2009; Zwietering et al. 2010). Where different industry sectors or FBOs are responsible for one or more steps in the food supply chain, the outcome at the point of consumption may rely on multiple food safety management systems that each govern product safety at an individual step in a food supply chain. Again, coordination and communication between the sectors/FBOs involved is then required for deciding on the appropriateness of the overall food safety management across the food supply chain at hand. Considering an individual step, as noted earlier, the food safety management system is then composed of one or more control measures that in concert exert the required level of control over the hazards entering that step for instance through raw materials, ingredients or from the operation's

environment. Both at an individual step as well as across steps so-called "system thinking" may help improve coordination of adequate control of product safety.

The food safety management system put in place for a single or multiple steps should be able to consistently meet the PO (or the FSO when the step concerned is the point of consumption), which will need to take into account possible variation in the hazard level coming into the food at the step. The change in hazard level between that coming into the step and the PO (or FSO) for that step is the so-called *Performance Criterion* (PC) (CAC 2007a). In effect, the PC is the net result of the control over a particular hazard achieved by the different control measures of a food safety management system at a step in the food supply chain. The operational implementation of the chosen food safety management system is through application of prerequisite programs and HACCP (Gorris 2005). These should include procedures that allow monitoring of operational parameters to verify process control, while taking into account process variability (see Chap. 13). In the case that specific knowledge about the ability of a food safety management system to actually meet the PO (or FSO) for a specific step is lacking, for instance when the underlying control measures cannot be based on sound science and/or validated, default values may be used that have proven to ensure food safety under other circumstances. Depending on the similarity between circumstances, the values chosen have an appropriate degree of conservatism, e.g., very low pH or a_w, or a severe heat treatment. A systematic overview of key properties of key pathogens possibly associated to foods has been previously published (ICMSF 1996).

Within the prerequisite programs and HACCP systems deployed, adequate auditing/inspection procedures and monitoring of key parameters should be ensured to verify the adequacy of operations to control microbiological, chemical and physical hazards relevant for the food and food operation and to meet a PO (or FSO) set for a microbiological hazard(s). Where appropriate, a *microbiological criterion* (MC) linked to a PO (or FSO) may be issued by a competent authority for the purpose of governmental monitoring and verification. Where governments establish such MCs, they are in principle risk-based criteria for lot acceptance or process control that identify non-conformance of lots or processes in terms of the prevailing governmental PO (or FSO) requirements. MCs should be established and applied only where there is a definite need and where application is practical (CAC 1997, 2013). For a range of commodities, ICMSF book 6 (ICMSF 1988, 2005) provides information that may be helpful to determine the relevance of establishing an MC for a particular pathogen. Examples of how MCs may be established to operationalize a PO or FSO using a risk based approach, can be found in Zwietering et al. (2015), which is part of a special volume of *Food Control* on the "Development of Microbiological Criteria for Food" (Caipo et al. 2015). MCs may also be established by industry for verification of ongoing control of their operation at a specific point in the food supply chain or for due diligence testing of final product acceptance. Other microbiological tests may be valuable for monitoring the microbiological status of the environment or of the product in the course of the processing/production operation ("in-process testing"). Detailed guidance on appropriate testing of ingredients, food processing environments, processing lines and finished products to enhance the microbiological safety and quality of the food supply can be found elsewhere (ICMSF 2011).

In cases where an FSO or a PO set by government is judged to be unachievable for an overall food supply chain or a specific step in that chain, respectively, options to consider include:

- governmental risk managers re-assess the FSO/PO level(s) established and to issue (a) level(s) that can technically be achieved or to select other/additional risk management options than FSO/PO to achieve the desired level of public health protection
- industry to consider bringing in new technologies or other operational capabilities not yet in place such that the FSO/PO level(s) is technically achievable or else to change the product and/or process design of the food product such that the hazard is sufficiently controlled
- the competent authority to ban the product from the market until new technologies, process capabilities, or alternative product formulations are available to enable meeting the FSO/PO

In an effort to make the concepts in food safety management underlying the new risk-based metrics more accessible, the commission published a layman-language guide (ICMSF 2010). This guide was first issued in 2005 in the English language and has since been translated into French, Portuguese, Spanish and Bahasa Indonesia as well as into the language of cartoons (see: www.ICMSF.org).

1.4 Historical Development of Modern Food Safety Management

Microbiological criteria for foods in international trade are addressed in the joint Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) food standards program, as implemented by the Codex Alimentarius Commission (CAC 1997, 2013). The program, established in 1962 (the same year as the establishment of the ICMSF), was the direct result of conflict between national food legislation and the general requirements of the main food markets of the world. Serious non-tariff obstacles to trade were caused by differing national food legislation. At that time, the Commission's objectives were to develop international food standards, codes of practice and guidelines, anticipating that their general adoption would help to remove and prevent non-tariff barriers to food trade.

Since the 1990s, risk analysis has developed within the Codex Alimentarius Commission (CAC) as the agreed framework for its work. In the process, FAO and WHO convened expert consultations for instance to address the three components of risk analysis (i.e., risk assessment, risk management and risk communication) and to develop specific guidance on the various stages of risk assessment (i.e. hazard characterization, exposure assessment and risk characterization). Risk analysis is now considered to be an integral part of the decision-making process of Codex. In 1999, the CAC adopted the Principles and Guidelines for the Conduct of Microbiological Risk Assessment (CAC 1999) and subsequently adopted the Principles and Guidelines for the Conduct of Microbiological Risk Management (CAC 2007a).

Codex develops and reviews standards and codes on an ongoing basis. Several aspects of food control are covered in Codex standards and Codes of Practice, including composition, labelling, additives, and hygiene. Subsidiary bodies of the Commission, the Codex committees, develop the standards and codes. Although the period from drafting a standard or code to its adoption at times is rather lengthy, the system has worked well, and many robust international food standards and codes of practice have been established. The Codex Committee on Food Hygiene (CCFH) has the major responsibility for all provisions on food hygiene in Codex documents, including microbiological criteria and codes of hygienic practice (effectively, good hygienic practices (GHPs)). The CCFH needs expert advice in dealing with highly specialized microbiological matters and especially in developing microbiological criteria. Over the 50 years of its existence, such advice has been extensively provided by the ICMSF through its many publications on sampling plans and microbiological criteria, HACCP, the principles for the establishment and application of microbiological criteria for foods, microbial ecology in foods and many other expertise areas relevant for Codex work.

The microbiological safety of food is principally assured by selection of raw materials, control at the source, product design and process control, as well as the application of prerequisite programs and HACCP during production, processing, distribution, storage, sale, preparation, and use. This comprehensive preventive system offers much more control than relying solely on end-product testing. Examples of measures that have successfully controlled foodborne hazards are given in Table 1.2.

Microbiological testing is time-consuming, often lacks sensitivity and specificity, and the levels of sampling routinely applied have a low probability of detecting defective lots when the proportion of defective servings within the lot is low (see Chaps. 6 and 7). Recognizing the limitations of end-product testing to ensure microbiological safety at port-of-entry, the ICMSF proposed a system of verification based on the use of prerequisite programs in combination with the HACCP system as a more reliable means of assuring product safety in the modern food industry (ICMSF 1988).

Table 1.2 Measures That Have Successfully Controlled Foodborne Hazards

	Hazard	Control measures	Other measures
Bacteria	B. cereus	Time-temperature control during cooking, cooling, and storage, retorting, acidification, low water activity	
	Brucella	Eradication of brucellosis	Animal health
	Campylobacter (thermophilic)	Raw material selection, avoid cross-contamination, cooking	GHP
	Cronobacter	Raw material selection, avoid recontamination, storage and consumer education	GHP, HACCP
	C. botulinum (proteolytic strains)	Retorting, acidification, low water activity	HACCP
	C. botulinum (non-proteolytic strains)	Cooking, time-temperature control, pH and a_w of products	HACCP
	C. perfringens	Time-temperature control during cooking, cooling, and storage	HACCP
	E. coli (pathogenic)	Cooking, controlled fermentation and aging, avoid recontamination	GHP
	L. monocytogenes	Cooking, controlled fermentation and aging, chilled storage, avoid recontamination	GHP
	M. bovis	Eradication of TB in cattle, pasteurization of milk	
	Salmonella (non-typhoid)	Cooking, controlled fermentation and aging, avoid recontamination	GHP
	S. typhi	Personal hygiene	
	Shigella	Cooking, water quality, waste water management, personal hygiene	
	S. aureus	Cooking, controlled fermentation and aging, chilled storage, avoid recontamination	GHP
	V. cholerae	Water quality, waste water management, personal hygiene	GHP
	V. parahaemolyticus	Raw material selection, avoid eating raw fish, cooking	GHP, HACCP
	V. vulnificus	Avoid certain foods, cooking	
	Y. enterocolitica	Separation of raw from ready-to-eat, extra hygiene at slaughter	GHP
Viruses	Hepatitis A	Water quality, personal hygiene, consumer education, cooking	Controls at harvesting
	Norovirus	Water quality, personal hygiene, consumer education, cooking	Controls at harvesting
Parasites	*Trichinella spiralis*	(at farms) limit access to farms, rodent control, freezing, cooking control at slaughter for anim als at risk (outdoor farmed pigs, game esp. wild boars), freezing, cooking	Animal health
	Toxoplasma gondii	Meat: freezing, cooking; vegetables: wash thoroughly	
Toxigenic fungi	*Aspergillus*	Raw material selection, sorting, dry storage, dehydration	GAP, HACCP
	Fusarium	Raw material selection, sorting, dry storage, dehydration	GAP, HACCP
	Penicillium	Raw material selection, sorting, dry storage, dehydration	GAP, HACCP
Seafood toxins	Ciguatera	Controls on harvesting, consumer education	GHP
	Scombroid poisoning	Hygiene, temperature control	GHP, HACCP
	Shellfish intoxication	Controls on harvesting, consumer education	GHP

Much of the information needed in HACCP to judge whether microorganisms grow, survive, or die during food processing, distribution, and use is contained in the scientific literature, but this information is not organized in a way that is convenient for those in the food industry needing to use that information. Hence, the ICMSF compiled published information that was judged by experts to be reliable in a series of easily used tables (ICMSF 1996). This was a conscious step towards promoting

the newly emerging concept of food safety management systems, based on Codex documents. The ICMSF then recognized that much of the information in ICMSF Book 3 (1980a, b) was out of date and did not consider the newly emerged pathogenic bacteria, such as *Listeria monocytogenes* and *Campylobacter jejuni/coli*, or many of the newer processes used in food manufacture. Consequently, the ICMSF updated its reviews of commodities in ICMSF Book 6 (1998b, 2005), but deliberately omitted consideration of sampling plans and microbiological criteria to emphasize that food management systems were replacing the earlier approach of end-product testing and offered better control of microbiological hazards.

That evolution of management systems continued with the first publication of Book 7 (ICMSF 2002) of which the current book is an updated edition. Book 7 aims to re-emphasize that end-product testing is merely one of several components of that, in concert, help to ensure food safety. Different types of sampling plans are considered, some more intensive than the "attributes plans" traditionally used at ports-of-entry, as they are intended for use when attempting to identify a problem and its source. Since production and processing methods and technical abilities continue to evolve at a rapid pace and new/re-emerging hazards reinforce the need for tailored food safety management of adequate stringency, ICMSF updated ICMSF Book 2 part 2 by publishing Book 8 (ICMSF 2011). Book 8 is linked to the commodity specific management considerations and emphasizes the benefits of various types of testing for food safety management other than end-product testing, such as environmental monitoring, in-line samples, shelf-life evaluation, etc. Over the years, ICMSF published various opinion documents and studies regarding modern food safety management, for instance on the use of epidemiologic data to measure the impact of food safety control programs as well as on the relationship between microbiological criteria and control measures with the new risk-based risk management metrics (ICMSF 2005; van Schothorst et al. 2009; Zwietering et al. 2010).

During the last 40 years, Codex Alimentarius Commission programs have gradually shifted from providing a basis for national standards to providing the point of reference in standards, guidelines and codes of practice for international trade. Subsidiary bodies of the CAC, the Codex committees, develop the standards and codes. Codex also, as a policy, adopted a sound-science and risk-based way of working in which expert bodies provide the risk assessment information and other pertinent scientific insight/data for the risk managers in the subsidiary bodies to make decisions when compiling standards, codes of practice or guidelines. At its 32nd session convened in 1999, CCFH identified 21 combinations of pathogens and commodities of significant public health concern. CCFH prioritized these according to criteria such as the significance of the public health problem, the extent of the problem in relation to geographic distribution and international trade and the availability of data and other information with which to conduct a risk assessment. CCFH suggested that FAO and WHO convene *ad hoc* expert consultations to provide advice on microbial risk assessment. This resulted in the creation of the Joint FAO/WHO Meetings on Microbiological Risk Assessment (JEMRA) as a key expert body for CCFH. JEMRA works under the auspices of FAO and WHO with the primary role to provide risk assessments and other relevant information to CCFH for consideration when CCFH develops its standards, codes and guidelines.

The charge to JEMRA for conducting risk assessments comes from CCFH in the form of a scoping document, risk profile or set of specific risk manager questions. Since its inception, JEMRA provided scientific advice on a broad range of pathogen and commodity combinations, including "Microbiological hazards associated with fresh produce," "Viruses in foods," "Enterohaemorrhagic *Escherichia coli* (EHEC) in meat and meat products," "*Salmonella* in eggs and broiler chickens," "*Listeria monocytogenes* in ready-to-eat foods," "*Vibrio* spp. in seafoods," "*Campylobacter* spp. in broiler chickens," "*Enterobacter sakazakii* and other micro-organisms in powdered infant formula," and "Foodborne parasites" (FAO/WHO 2015).

Much of the work done by CCFH since 2000 has received JEMRA input, either through their international risk assessments or expert consultations, in addition to input from specialist observer organizations such as ICMSF. The risk-based Codex guidance developed since that time includes: "Principles and Guidelines for the Conduct of Microbiological Risk Management" (CAC 2007a), "Code of Hygienic

Practice for Eggs and Egg Products" (CAC 2007b), "Guidelines on the Application of General Principles of Food Hygiene to the Control of *Listeria monocytogenes* in Ready-to-Eat Foods" (CAC 2007c), "Code of Hygienic Practice for Powdered Formulae for Infants and Young Children" (CAC 2008b), "Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic *Vibrio* Species in Seafood" (CAC 2010), "Guidelines for the Control of *Campylobacter* and *Salmonella* in Chicken Meat" (CAC 2011), "Guidelines on the Application of General Principles of Food Hygiene to the Control of Viruses in Food" (CAC 2012), "Principles and Guidelines for the Establishment and Application of Microbiological Criteria Related to Foods" (CAC 2013a), "Guidelines for the Control of *Taenia saginata* in Meat of Domestic Cattle" (CAC 2014) and "Guidelines for the Control of *Trichinella* spp. in Meat of Suidae" (CAC 2015). As an "international non-governmental observer (INGO)" to CCFH, ICMSF has played an active role in the development of these international standards.

1.5 Foodborne Illness: Etiologic Agents and Contaminants

1.5.1 Bacteria

In industrialized countries, and based on reports from those that have carried out comprehensive assessments of the number of illnesses, hospitalizations and deaths from contaminated foods, the most common causes of foodborne illness are norovirus, *Salmonella enterica*, *Campylobacter* spp., *Clostridium perfringens* and *Staphylococcus aureus*, (Scallan et al. 2011a, 2013; Adak et al. 2002; Hall and Kirk 2005). Due to its high case fatality rate, *L. monocytogenes* has also become a much noted cause for concern. Scallan et al. (2011a) reported that norovirus was responsible for most (58%) foodborne illness in the US, while non-typhoidal *Salmonella* was the leading cause of hospitalizations and mortality. Collectively, *Campylobacter* spp., non-typhoidal salmonellae, *Clostridium perfringens*, *L. monocytogenes* and *E. coli* O157, norovirus and *Toxoplasma gondii* accounted for 90% of all domestically-acquired cases of foodborne illnesses, hospitalizations and deaths in the U.S. (Scallan et al. 2011a). Attention has also been drawn to *Cronobacter* spp., possibly associated with powdered infant formulae and linked to fatalities and sequela in neonates.

A relatively low proportion of illnesses have been traced back to particular foods. Where that has been possible, the common contributory factors are very similar in different countries. For example, inadequate thawing prior to cooking occurred on numerous occasions with large Christmas turkeys. The subsequent cooking failed to kill salmonellae in the center of the partially thawed bird, resulting in salmonellosis. Inadequate temperature control after cooking includes preparation too far in advance of consumption; storing at warm ambient (non-refrigeration) temperatures; and attempting to cool large amounts of heated food that cannot be cooled quickly, resulting in growth of surviving bacteria during the slow cooling, and consumption without reheating above ca 63 °C to kill the vegetative cells of, e.g., *Bacillus cereus* in rice or *Clostridium perfringens* in meat or gravy. Contamination of a cooked, safe food with microorganisms from raw food is another important factor causing foodborne illness, e.g., at barbecues.

Salmonellae have been considered the most important foodborne pathogenic bacteria worldwide for many years, and are estimated to cause approximately 94 million cases of non-typhoidal gastroenteritis each year (Majowicz et al. 2010). In many countries, the most commonly implicated salmonellae have been *Salmonella* Enteritidis [*Salmonella enterica* serovar Enteritidis] and *Salmonella* Typhimurium [*Salmonella enterica* serovar Typhimurium]. A great number of different foods and food ingredients have been implicated in outbreaks of salmonellosis. Frequent food vehicles for salmonellae include meat and poultry, eggs and egg products, milk and milk products, fresh produce and unpasteurized juices (de Roever 1998) as well as spices. In many countries, the occurrence of *S.* Enteritidis particularly in poultry and in eggs increased greatly from the mid-1980s to become a serious problem, but outbreaks linked to this pathogen declined in the UK from 1996–1997 (ACMSF 1993, 1996, 2001) and continued to decrease up to 2012 (PHE 2014).

In recent years, the incidence of disease caused by thermophilic *Campylobacter* spp. has increased prominently in many industrialized countries (Friedman 2004). For instance, *Campylobacter* is the second most common bacterial cause for hospitalization associated with foodborne illness in the U.S. (Scallan et al. 2011a), and it is the leading bacterium responsible for infectious intestinal illness in the U.K. (Tam et al. 2012). Outbreaks of campylobacteriosis are rare, with most cases being sporadic and commonly attributed to undercooked poultry or cross-contamination from raw poultry. Other foods, untreated water, and raw milk also have been implicated (Altekruse et al. 1999).

In countries where raw fish is an important part of the diet, disease caused by *Vibrio parahaemolyticus* is frequent. Occasional outbreaks occur in western countries, but there the vehicle of transmission is usually processed rather than raw seafoods. Notably, consumption of raw oysters contaminated with *Vibrio vulnificus* pose significant concern for immunocompromised individuals. *Vibrio cholerae* is endemic in many tropical countries, and water plays a major role in the epidemiology of cholera.

Shigella spp. also represent an important public health problem in many developing countries and cause significant illness in developed countries. Cases of shigellosis reported in developed countries are often associated with travelling, food handlers, and day care centers. Because the reservoir of *Shigella* spp. is restricted to humans, the source of infection is food or water contaminated by human carriers.

Disease caused by *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* occurs worldwide and has been primarily associated with the consumption of raw or undercooked pork and produce held at refrigeration temperatures for extended periods.

Escherichia coli strains are a common part of normal microbial flora of animals, including humans. Most strains are harmless, but some cause diarrhea. Strains carrying particularly virulent properties have emerged as a serious hazard, with consumption of even low numbers of these organisms bearing a risk for life-threatening illness. During the last three decades, enterohaemorrhagic *E. coli* (EHEC), producing verocytotoxins (VTs), also called Shiga toxins (STs), have emerged as a serious foodborne hazard. Many different serotypes of *E. coli* produce VTs or STs. Initially, most human outbreaks of EHEC were due to *E. coli* O157:H7, although serogroup O111 is the most common cause of illness in Australia. Human illness has now been associated with many verocytotoxin-producing serotypes of *E. coli*, with serotypes O157, O26, O45, O103, O111, O121, and O145 being responsible for some 90% of illnesses. Human cases of EHEC were initially associated with consumption of undercooked ground beef (Bell et al. 1994) and, occasionally, unpasteurized milk. However, fresh fruits and vegetables contaminated with EHECs are being implicated in a growing proportion of outbreaks (Sivapalasingam et al. 2004; Mahon et al. 1997; EFSA 2007; FAO/WHO 2008b; Lynch et al. 2009; Mathusa et al. 2010; Beutin and Martin 2012; Soon et al. 2013; Olaimat and Holley 2012). Outbreaks have also been traced to unpasteurized apple juice ("apple cider" in the U.S.), yogurt, fermented sausage, water, and contact with farm animals (Doyle et al. 1997; Mathusa et al. 2010). Of particular note is the major outbreak in Europe involving Shiga toxin-producing *E.coli* (STEC) O104:H4 present in fenugreek spouts (Beutin and Martin 2012; EFSA 2012; Soon et al. 2012), with this strain also sharing virulence characteristics with enteroaggregative *E.coli* (EAEC).

Disease caused by *L. monocytogenes* is not frequent, but can be severe, with a high mortality rate in populations at risk, such as infants, pregnant women, and the immunocompromised. The bacterium is ubiquitous and foods implicated in outbreaks range from products made from raw milk, butter, ready-to-eat meat products, surimi, smoked mussels and trout, fruits and vegetables. Two major outbreak of listeriosis for instance occurred in Canada in 2008, one major outbreak being linked to ready-to-eat meat cuts and another one to the consumption of cross-contaminated pasteurized milk cheese (Gaulin et al. 2008; PHAC 2008). Two other significant public health events in the U.S. linked to *L. monocytogenes* were an outbreak of listeriosis attributable to contaminated cantaloupe and milk shakes made from contaminated ice cream (CDC 2012, 2015).

Specific international attention has been given to the safety of food for infants and young children as it relates to the possible presence of *Cronobacter* spp. (previously referred to as *Enterobacter sakazakii*) in powdered formulae (Norberg et al. 2012; Yan et al. 2012). *Cronobacter* spp. has been docu-

mented as a rare cause of outbreaks and sporadic cases of neonatal meningitis, necrotizing enterocolitis, and sepsis in infants with a high mortality. They are ubiquitous in nature, and have been found to occur in different food operations as well as in consumer homes (Kandhai et al. 2004). Among infants, those at greatest risk are infants less than 2 months of age, particularly pre-term infants, low birth weight infants (<2500 g), and immunocompromised infants. FAO and WHO have established two international risk assessments on the topic (FAO/WHO 2006b, 2008a). Other than powdered infant formulae, no food source has specifically been epidemiologically linked to disease caused by *Cronobacter* spp. and therefore the focus of food safety management has been on the potential presence of these pathogens in processing facilities for infant formulae, raw materials and finished products.

Botulism occurs relatively infrequently, but remains a serious concern because of the life-threatening nature of the disease and the impact on trade of the incriminated product type. For many years, commercially processed foods were less frequently involved than home-canned or home-prepared foods, but more recently several commercially prepared products have been implicated. Faulty processing and/or inappropriate storage temperatures have been the most common reasons for botulism, with home-processed foods and foods mishandled in food service establishments also responsible. Foods implicated include hot sauce containing jalapeño peppers prepared without adequate heating, potato salad prepared with foil-wrapped baked potatoes previously stored at room temperature, sautéed onions in butter stored unrefrigerated and then served on a sandwich, tiramisu-mascarpone cheese under-processed canned chili and non-refrigerated carrot juice. With improved refrigerated storage during food distribution and use, *Staphylococcus aureus* and *Clostridium perfringens* now cause illness only when there has been temperature abuse. Due to improved refrigeration, however, the shelf-life of many foods has lengthened possibly leading to a new concern that psychrotrophic pathogens may increase to dangerous levels without spoilage being evident to the consumer. Microorganisms of most concern in this regard are non-proteolytic strains of *C. botulinum* types B, E, and F, *L. monocytogenes* and *Y. enterocolitica*, all of which cause little or no deterioration of the food supporting their growth.

Other foodborne bacterial pathogens include *Streptococcus pyogenes*, *Mycobacterium tuberculosis*, *Brucella abortus*, and *Bacillus cereus*. An overview of relevant pathogens for a range of food commodities has been compiled (ICMSF 1998b, 2005).

1.5.2 Viruses

Hepatitis A virus (HAV),norovirus (NoV) (formerly known as Small Round Structured Viruses, or SRSVs, or Norwalk/Norwalk-like viruses),and rotavirus are known causes of foodborne illness. As methods of detection have improved and national initiatives on surveillance and ascertainment are being intensified, NoV has been recognized more often as the most important cause of non-bacterial gastroenteritis throughout the world (Caul 2000). Viruses are occasionally involved in large outbreaks (Weltman et al. 1996), but the true extent and importance of viruses in foodborne illness has not been adequately assessed (ACMSF 1995). Studying the burden of foodborne illnesses in the USA, Mead et al. (1999) already estimated that viruses are more important than bacteria and protozoa as a cause of foodborne illness. This was confirmed by more recent data (Scallan et al. 2011a) establishing that norovirus alone is responsible for 58% of illnesses, as compared to 30% in total being caused by the top three bacterial pathogens (i.e., non-typhoidal *Salmonella* spp., *C. perfringens*, and *Campylobacter* spp.). Next to being the leading cause of foodborne illness, NoV was identified as the cause of second highest number of hospitalizations and the fourth highest cause of death (Scallan et al. 2011a). Surveillance of infectious intestinal illness data from the UK suggest that norovirus, sapovirus and rotavirus are the leading causes there (Tam et al. 2012). In Australia, NoV was identified as the

leading cause of foodborne illness, accounting for 30% of illnesses (Hall and Kirk 2005). Live bivalve molluscs are often implicated in viral foodborne disease outbreaks (Halliday et al. 1991). A review of food- and waterborne outbreak events due to NoV between 2000 and 2007 revealed that in 42.5% of the cases the food handler was responsible for the outbreak, contaminating sandwiches and catered meals, followed by water (27.5%), bivalve shellfish (17.5%) and fresh produce (12.5%) (Baert et al. 2009). A review of the global burden of disease and of particular viruses of concern determined that NoV and HAV are most frequently involved in foodborne viral infections (FAO/WHO 2008c), based on the symptoms of infection, these viruses can be grouped into those that cause gastroenteritis (NoV, human rotavirus, astroviruses, Aichi virus, Adenoviruses and Sapoviruses), enterically transmitted hepatitis (caused by HAV and Hepatitis E virus, which migrate to the liver, where they manifest disease), and a third group which replicates in the human intestine, but only cause illness after they migrate to other organs such as the central nervous system (Enterovirus).

1.5.3 Protozoa

Foodborne protozoa have also been incriminated in large outbreaks, e.g., *Cryptosporidium parvum* from apple juice and water (Osewe et al. 1996), and *Cyclospora cayetanensis* from raspberries, lettuce, and water (Speer 1997). In immunocompromised persons, diarrhea may be severe, making the illness serious and difficult to treat. Large outbreaks in North America have had a huge impact on international trade in soft fruit and salad vegetables because if present on the initial crop, these protozoa are almost impossible to eliminate. Illness due to *Toxoplasma gondii* is also much more serious in immunocompromised persons and pregnant women. In the USA, *T. gondii* is an important cause of hospitalization and death in the US (Scallan et al. 2011a) and a major economic burden among the foodborne diseases, due to deaths and life-long impairments of infected infants (Batz et al. 2011). Sporadic cases of protozoal infection have also been linked to consumption of undercooked meat, primarily pork, and fish products.

1.5.4 Seafood Toxins

Disease caused by histamine and other biogenic amines can arise from several foods, notably scombroid fish species. In North America, illness attributed to histamine is the second most common disease from fish, excluding shellfish (MMWR 2000).

The principal intoxications having a microbiological origin in seafood include paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP), amnesic shellfish poisoning (ASP) (also known as domoic acid poisoning), ciguatera, and scombroid (histamine) fish poisoning. PSP, DSP, and NSP are caused by toxins produced by dinoflagellates, and ASP by a diatom. All these diseases typically result from consumption of bivalve molluscs that have been feeding on the toxigenic algae. The toxin(s) causing Ciguatera are derived from toxigenic microalgae growing in and around tropical coral reefs and passed up the marine food chain through herbivorous reef fish to more far-ranging carnivorous species. Humans typically become intoxicated from eating the toxic fish. Histamine or scombroid poisoning is caused by consumption of fish containing high levels of histamine (and other biogenic amines) resulting from histidine dehydrogenase activity of bacteria multiplying on the fish after death. With the exception of histamine and other biogenic amines, toxin accumulation is passive. All the seafood toxins are resistant to heating and, therefore, cannot be destroyed by cooking. They are undetectable organoleptically (ICMSF 1996; Liston 2000; Whittle and Gallacher 2000).

1.5.5 Toxigenic Fungi

Mycotoxins are fungal metabolites which when ingested, inhaled or absorbed through the skin, can cause disease or death in man and animals. Although there are many types of toxic metabolites, most are of little concern for humans, either because their toxicity is limited or because they are produced by species that are uncommon in foods. The most important mycotoxins are aflatoxins (B_1, B_2, G_1 and G_2), ochratoxin A, fumonisins, deoxynivalenol, zearalenone (Miller 1995) and patulin. These toxins are produced by a few species of fungi from the common genera *Aspergillus*, *Penicillium* and *Fusarium*. These fungi may grow and produce toxins on the crop before harvest or after harvest during drying and storage. The production of mycotoxins also depends on a wide variety of agronomic and climate conditions as well as on whether a particular cultivar is grown within the area to which it is adapted (Pitt et al. 2012; Taniwaki and Pitt 2013).

On a global scale, the most important mycotoxins are the aflatoxins that are produced by *Aspergillus flavus, A. parasiticus, A. nomius* and related species, growing mainly in peanuts, tree nuts, cotton seed and maize. Aflatoxins have a likely involvement in five toxic effects: acute toxicity, liver carcinogenicity, growth retardation in children, immunosuppression and liver cirrhosis. Aflatoxins cause primary liver cancer in humans and outbreak cases and deaths related to consumption of food contaminated with aflatoxins have been reported, especially in developing countries (Azziz-Baumgartner et al. 2004; Lewis et al. 2005; Taniwaki and Pitt 2013). Ochratoxin A is a chronic nephrotoxin, affecting kidney function in all animal species tested, and probably has a role in kidney disease in wide areas of Europe. Ochratoxin A is produced by three well defined groups of fungi: firstly, the ochre coloured aspergilli, comprising *Aspergillus ochraceus, A. westerdijkiae, A. steynii* and a few other related species; secondly, the black aspergilli, *A. carbonarius* and common species *A. niger*, which produces OTA only infrequently; and, thirdly, the *Penicillium* species, *P. verrucosum* plus the closely related species *P. nordicum* (Frisvad et al. 2006; Pitt and Hocking 2009). Another role in human disease is probable for fumonisins, produced by *Fusarium verticillioides* and *F. proliferatum* during growth in maize, which have been implicated in human esophageal cancer. It has been shown that some fumonisins are also produced by *Aspergillus niger*, an entirely unexpected source (Frisvad et al. 2007). *Aspergillus niger* has usually been regarded as a benign fungus and has been widely used in enzyme production and ingredients for food processing. It holds GRAS (generally regarded as safe) status and is among the most commonly reported from foods. The possibility of co-occurrence of ochratoxin A and fumonisin B_2 in foods is of concern (Taniwaki and Pitt 2013). Trichothecene toxins, such as deoxynivalenol and nivalenol produced by *Fusarium graminearum* and related species in cereals, cause immunosuppresssion and consequently have a potentially important role in reducing disease resistance, which as yet is ill-defined. Zearalenone is an oestrogenic mycotoxin and is produced by the same *Fusarium* species that produce DON and nivalenol, and generally under the same conditions; the main sources are maize and small grains. Patulin is produced mainly by *Penicillium expansum* and *P. sclerotigenum* and the occurrence of this toxin is related to apple products. Patulin shows neurotoxic activity for certain animals but in humans, it is still unclear whether there are harmful effects.

1.6 Surveillance and Trends in Foodborne Illness

The symptoms of foodborne illness range from mild to severe gastroenteritis, to life-threatening disease. Foodborne disease is commonly acute, but also can become chronic with long-term sequelae including organ failure, paralysis, neurological impairment, blindness, stillbirths and death.

Although foodborne infections have been recognized as a major cause of human illness for many years, the true incidence often remains unknown (Motarjemi and Käferstein 1997; Clark et al. 2000) or the attribution of illness to specific sources is a challenge. As a consequence, the attributable risk is

difficult to quantify too. In Canada and the US, for example, the number of cases and outbreaks that actually occur is estimated to be many times greater than the reported figures according to several studies conducted in the last couple of decades (Todd 1996, 1997; Buzby and Roberts 1997; Mead et al. 1999; Scallan et al. 2011a, b). Studies of infectious diseases estimating the extent of underreporting include Notermans and Hoogenboom-Verdegaal (1992) for the Netherlands, Wheeler et al. (1999) and FSA (2000) for England, and Hall et al. (2005a) for Australia.

Attribution of foodborne disease is difficult; a recent study specific to the USA situation, reported that almost 75% of foodborne illnesses could not be attributed to a specific pathogen (Scallan et al. 2011a). Currently there is wide variation in the approaches used to attempt to estimate attributable risk (Batz et al. 2005; EFSA 2008; ICMSF 2006; Pires et al. 2009). Examples of different approaches include the analysis of outbreak data, case-control studies, microbiological sub-typing, source-tracking methods and expert judgment. Considerable effort is now being made to determine the relative contributions to illness from different sources and vehicles of human infection. However, a method appropriate in one country may be unsuitable in another. This very much depends on the population, infrastructure for surveillance and/or monitoring, resources available for healthcare, laboratory facilities for sub-typing isolates from animals, human cases and food samples.

Underreporting is unfortunate and the difficulty to confidently assess the magnitude of under reporting further impacts on the accuracy of the available data. Next to providing a continuing assessment of trends in etiological agents and food vehicles, the utility of timely incidence data covers a range of aspects relevant to the management of food safety and the protection of public health, including: (a) identification and removal of contaminated products from the market, (b) correction of faulty food-preparation practices in food-service establishments, processing plants, and homes, (c) identification and appropriate treatment of human carriers of foodborne pathogens, (d) possible detection of new agents of foodborne disease, (e) a better understanding of the effectiveness of regulatory policy and/or its implementation, and (f) improved understanding of the epidemiology of various pathogens and identification of strategies to intervene with their transmission.

Periodically, overviews summarize trends in foodborne disease (Bean et al. 1990, 1997; CAST 1994, 1998; POST 1997; WHO 1997; Mead et al. 1999; Adak et al. 2002; Flint et al. 2005; WHO 2009, 2014; Batz et al. 2011; Scallan et al. 2011a, b, 2013; EFSA/ECDC 2012). Nevertheless, assessing the true global burden of foodborne illness remains a challenge being tackled by WHO through its Foodborne Disease Burden Epidemiology Reference Group (FERG) project (WHO 2009, 2014). What comes across from the available insights is that illnesses caused by foodborne pathogens constitute a worldwide public health problem influenced by demographics, industrialization and centralization of food production and supply, travel and trade, and microbial evolution and adaptation (Tauxe 1997; Quested et al. 2010). For the USA only, Scallan et al. (2011a) estimate 9.4 million cases of illness caused by 31 different known pathogens, including viruses and protozoa next to an estimated 38.4 million of cases due to unspecified foodborne agents.

1.7 Importance of Effective Control Measures

To assess what food safety management system needs to be put in place, it is necessary to evaluate the product and process design, the intended user/consumer, possible unintended use, etc., and to verify that relevant food safety standards in law or industry practice will be met on an ongoing basis. This holistic "systems thinking" will lead to the selection of appropriate control measures and the stringency with which these are to be applied. *Control measures* are actions and activities that prevent or eliminate a food safety hazard or reduce it to a tolerable level. One or more control measures may be necessary at each stage along the food chain to assure that a food is safe when consumed. Effective food safety management systems almost always comprises a number of different control measures,

such as raw material selection, storage under controlled conditions, hygienic handling prior to a process, correct application of the process, and prevention of post-process contamination (see Chap. 3). The suite of control measures are managed within prerequisite programs and HACCP systems. In ICMSF Book 4 (1988), critical control points (CCPs) were divided into those control measures that eliminate the hazard, CCP1, and those that can reduce but that cannot prevent or eliminate the hazard, CCP2. Many HACCP plans would be better understood if that differentiation were reintroduced.

To control *Campylobacter* and *Salmonella* at the relevant stages of the broiler supply chain, some measures should be considered, such as the use of ASC (acidified sodium chlorite) in on-line reprocessing (OLR), crust freezing, high oxygen concentration during chilled storage. Also, air chilling used to reduce carcass temperature and forced air chilling (blast chilling) will be effective for reducing the risk of *Campylobacter*. For control of *Salmonella* in eggs, vaccination of laying hens has been shown to be an effective control measure in reducing the prevalence of *Salmonella* in eggs and this has contributed to a reduction in salmonellosis cases linked to eggs in the UK.

Some food products are particularly vulnerable to microbiological contamination from primary production due to the subsequent survival and/or growth of pathogens, and the lack of a processing step to eliminate these. Among these products are fresh and fresh-cut fruits and vegetables for which good agricultural practices are a key basic control measure, next to considerations of other possible controls at later stages (IFT/FDA 2001; FAO/WHO 2008b). A major incident with a rare strain of *E.coli* O104:H4 in Europe in 2011 was traced back to contaminated fenugreek seeds (Beutin and Martin 2012) and again highlighted the importance of proper control of contamination at the stage where sprout seeds are produced and for proper hygiene and final preparation as control measures along the food chain.

At the industrial level, heat processes have been developed for low-acid canned foods to control *C. botulinum* spores. Less severe heat processes are adequate for acid or acidified foods (pH 4.5 or below) because *C. botulinum* spores are unable to multiply at those low pH values. Spores of *C. perfringens* in beef survive most cooking processes and are able to multiply if the cooked meat is held at suitable temperatures. Growth is minimized by cooling quickly through the range of temperatures supporting growth (50 °C down to 15 °C). For fruit juices (e.g. apple juice) that may be contaminated with *E. coli* O157:H7, and fermented meats using ground beef that may be also be contaminated with *E. coli* O157:H7, these should undergo treatments that eliminate the hazard.

At the catering level, other controls can be applied. *Escherichia coli* O157:H7 occurs sporadically in ground (minced) beef used in hamburgers as well as in vegetables. Heating sufficiently will eliminate the hazard, and appropriate heat processes have been developed to ensure that the center of the meat patty reaches a temperature that is lethal to pathogenic *E. coli*, taking account of the weight, thickness, and initial temperature of the patty, the temperature of the grill, and the duration of cooking.

At the consumption level, the safety of ready-to-eat food could be achieved by control measures such as better temperature control or limiting the length of storage periods will mitigate increased risk due to increases in *L. monocytogenes*. The vast majority of cases of listeriosis are associated with the consumption of foods that do not meet current standards for *L. monocytogenes* in foods, whether that standard is "zero tolerance" or a microbiological limit such as 100 CFU/g. Monitoring and controlling refrigerated storage temperatures are key control measures. The product temperature should not exceed 6 °C (preferably 2–4 °C). Temperature abuse that may occur supporting the growth of *L. monocytogenes* could result in a reduction of product shelf life.

The length of the shelf-life is another important factor contributing to the risk associated with foods that support *L. monocytogenes* growth. The shelf-life of such foods should be consistent with the need to control the growth of *L. monocytogenes,* since *L. monocytogenes* is able to grow at refrigeration temperatures. Even the appropriate low temperatures may not be maintained throughout the entire food chain until the point of consumption.

To prevent salmonellosis from *S.* Enteritidis in eggs at the domestic level, vulnerable persons, such as the elderly and the immunocompromised, should consume only eggs that have been cooked until the yolk is solid, or use a commercially pasteurized product. Numerous cases of salmonellosis have been traced to using raw eggs in homemade mayonnaise and tiramisu. Handling raw poultry in the kitchen can spread thermophilic *Campylobacter* spp. to other working surfaces. Hands should be washed frequently and thoroughly after handling raw poultry.

For foods intended for vulnerable consumers, all industry sectors/ FBOs involved in the chain from production to consumption have a role to play in terms of overall control of product safety. For instance, in the case of powdered infant formulae (PIF), producers, manufactures, distributors, transporters, retailers, as well as caregivers need to achieve an effective continuum for the purpose of reducing risk of *Cronobacter* spp. Amongst others, producers and manufacturers of raw materials should ensure that good agricultural, hygienic and animal husbandry practices are employed at the farm level. Manufacturers of ingredients and packaging materials should utilize good manufacturing and good hygienic practices and have HACCP systems implemented. PIF manufacturers should provide additional measures to control hazards in the formulae during and after reconstitution. Distributors, transporters and retailers should assure that the product is handled and stored properly. Hospitals and institutions should establish hygienically designed rooms designated for preparation of formulae and that good hygienic practices are followed. Professional caregivers (as well as parents in home situations) should ensure that PIF is prepared, handled and stored properly and according to the manufacturers' instructions.

1.8 Effectiveness of Prerequisite Programs and HACCP

HACCP has proven very effective as a control system for many food processes, especially those with a step that eliminates the hazard (e.g., canning of low-acid foods, cooking to eliminate salmonellae or *L. monocytogenes*), or foods that are formulated to prevent microbial multiplication (e.g., low a_w, low pH). Of greater concern are processes that during production or before consumption do not have a step that can prevent or eliminate a known hazard, for instance raw vegetables or ready-to-eat foods, respectively.

Frequent reports on foodborne illnesses and incidents occurring in the media around the globe as well as the instant distribution of information via the internet and social media have fueled the public perception that food safety is declining. Although it needs to be recognized that absolute safety (i.e., "zero risk") cannot be guaranteed, today's food safety systems are stronger than ever before due to better implementation of prerequisite programs and HACCP. Where records are available, the majority of past recalls are due to failures to adhere to particular prerequisite programs, rather than in failures of plans implementing prerequisite programs or HACCP. In addition improved laboratory and detection methods allow better recognition of potential problems, possible agents, and hazard-food combinations. Hence the safety of foods is improving, despite recognizing new agents and new hazard-food combinations. Some new pathogens force reconsideration of traditional control measures, e.g., VT-producing *E. coli*, present sporadically and usually in low numbers in cattle, are difficult to detect and control. When new agents are recognized, government and the food industry respond to control them, but it must be recognized that it may take time to understand the conditions leading to illness and the changes necessary to control them.

The current trends in food processing (i.e. to reduce the extent of heating, to minimize the use of chemical preservatives, and to provide foods that require little or no preparation or are ready-to-eat and consequently not subjected to heating prior to consumption) all increase the likelihood of pathogens reaching the consumer. Even with the greatest care in agricultural practices, it is not possible to eliminate all pathogens from raw agricultural and seafood commodities.

1.9 Would PO/FSO Improve Food Safety and Reduce Foodborne Illness?

Although HACCP plans are now more widely implemented, the main weaknesses are that the required "stringency", i.e. the required level of control over the relevant hazard(s) needed to make a food safe, is not stated clearly and there is little or no guidance on what is expected of an adequately designed and implemented HACCP plan. This omission is widespread in documents prepared by Codex, many advisory groups, and in governmental regulations. An FSO would be a metric that specifically indicates the level of control needed over a particular hazard associated to a food in order to establish adequate prerequisite programs and HACCP systems.

A further issue is the continued indiscriminate use of microbiological testing of "finished" or end products. That testing is usually inadequate in terms of sampling and the number of samples tested, and often poorly targeted in terms of the hazard(s) most likely present in the particular product. With the introduction of HACCP as an essential building block of an effective food safety management system on top of prerequisite programs in the 1990s, there has been no decrease in end-product testing that might have been expected with increased control. If anything, such testing has continued to increase with little appreciation of the limited value that this provides.

1.10 Use of PO/FSO in Food Safety Management

What constitutes safe food is a societal decision that is in the hands of politicians and risk managers of the competent authority or authorities governing food safety in a country and who need to consider public health impact, technological feasibility, economic implications, and other risks in everyday life. In essence, the safety of a particular food or food category relates to the level of risk that is deemed "tolerable" or "acceptable" for this food in the country concerned. Thus, safe food is not necessarily "zero-risk" food or food "with no hazard in it at all". Notably, risk is an estimate of the probability and severity of the adverse effects in an exposed population that may result from a hazard in a food. Even when a particular hazard is present in a food, in the case that there is a very low chance of exposure of a vulnerable population, the risk may be acceptable. When considering the level of acceptable/tolerable risk for a given hazard-food combination, risk managers should seek input from risk assessors and consult stakeholders, such as the affected industry and consumers. When decisions have been made on the level of a risk that can be accepted/tolerated, this could take the form of an ALOP, i.e. a number of cases per unit of population in a country that signifies the achieved or achievable level of public health protection.

Food operators cannot address an objective such as an ALOP that states, for example: "there shall be no more than 20 domestic cases of a certain foodborne illness per 100,000 inhabitants per year in a country" as an expression of the tolerable level of risk. While this may be a desirable goal, it requires the collective efforts of many parties. Food operators can only address factors over which they can exercise control, which in principle are the microbiological, chemical and physical hazards possibly associated with the food raw material or product they handle or produce at the particular step in the value chain that they are involved in. While all operators along the food chain must understand their role and manage their operation to ultimately satisfy an FSO, they cannot assume responsibility for the actions of all others along the food chain. It is important that each FSO clearly communicates the level of hazard that is considered tolerable at the point of consumption in such a manner that food operators (or regulators) can effectively establish an appropriate performance objective (PO) for the step that can be controlled and derive the performance criterion (PC) to meet the PO. Whenever possible, FSOs and POs should be quantitative and verifiable as this specifically strengthens communication of responsibilities along the food chain. However, this is not always possible given data lacks

regarding for instance relevant properties of the hazard, factors that lead to adverse public health effects, conditions necessary to control the hazard, and/or control measures that can be implemented effectively in the food chain. This is a common situation particularly with newly recognized or emerging hazards, e.g., VT *E coli*. As more information becomes available, risk assessments should be updated and FSOs and POs adjusted accordingly and expressed more quantitatively.

Supported by risk assessment and risk management processes (CAC 1999, 2007a, b, c), FSOs and POs can play an important role in modern food safety management systems by linking information from the level of public health protection to adequate measures to control identified risk(s). Basic information is provided in Chap. 3 that can be used to establish scientifically based control measures.

For foods in international commerce, FSOs should be developed within the Codex framework. This is consistent with the concepts of the World Trade Organization and its SPS agreement, which provides a framework for harmonization of acceptance criteria for foods in international trade. Food safety criteria developed in one country frequently differ from those of other countries. The principles presented in this text can lead to a scientific basis for comparing the relative level of protection afforded by different food safety systems. These principles are applicable to issues of equivalency, levels of protection, and non-tariff trade barriers. Their application should facilitate the harmonization of international trade where the practices of one country may differ from those of another, yet the practices of both countries provide safe products. Furthermore, they can be applied by control authorities and food operators for the establishment of equivalent criteria.

FSOs are a preferred approach to food safety management because the concept focuses on protection of human health while offering flexibility in achieving that goal. FSOs and POs specify goals that can be incorporated into the design of control measures (e.g., prerequisite programs, HACCP) for the production and preparation of foods. They can also provide the basis for assessing the adequacy and effectiveness of control systems adopted by industry and inspection systems adopted by regulatory authorities. Importantly, FSOs and POs are limited to food safety and do not address quality.

POs are generally considered more specifically appropriate for competent authorities to verify food safety expectations and microbiological criteria could be established for this purpose (Zwietering et al. 2015). Notably, FSOs and POs differ from microbiological criteria by communicating the level of hazard that is considered tolerable for consumer protection, but they do so without detailing how these objectives are to be met and how meeting these are to be verified. Microbiological criteria would be the tool of choice for verification when microbiological testing plays a role in this (CAC 2013). Microbiological criteria can be developed and applied for different purposes and in different situations, as discussed in a series of papers (Caipo et al. 2015) developed under auspices of FAO and WHO to support the revision of the "Principles and Guidelines for the Establishment and Application of Microbiological Criteria Related to Foods" (CAC 2013).

1.11 Performance, Process, Product, and Default Criteria

When designing and controlling food operations, it is necessary to consider pathogen contamination, destruction, reduction, survival, growth, and possible recontamination. Consideration should also be given to subsequent conditions to which the food is likely to be exposed, including processing and potential abuse (time, temperature, cross-contamination) during storage, distribution, and preparation for use. The ability to control these factors and conditions at each stage in the food chain to prevent, eliminate, or reduce food safety hazards varies with the type of food and the effectiveness of available technologies. Since prerequisite programs and HACCP are the primary tools available to industry to control microbiological hazards in food operations, it is essential that the technical

achievability of the FSO and related PO(s) is confirmed. The "hierarchy" of risk-based metrics from ALOP at population level to control measures at industry level has been described by Codex Alimentarius (CAC 2007a).

A *Performance Criterion* (PC) is the required outcome of one or more control measures at a step or combination of steps that contribute to ensuring the safety of a food. The outcome can be an overall reduction in the hazard level or a limited increase or no-change. When establishing a PC, account must be taken of the initial level of the hazard, potentially coming from different sources, and changes in the level of the hazard as a result of handling, production, processing, storage, cross-contamination, etc., during the step that delivers the PO (or the FSO when the step is the final one before consumption). In other words, a PC describes the outcome that is needed to achieve a particular PO at a specified point in the food chain or to achieve the FSO when it concerns the point of consumption. PCs are established by individual food businesses in most cases, but competent authorities may specify specific control measures as default guidelines where the application of a control measure is generally uniform. An example of a PC is an overall 6D kill of salmonellae and prevention of recontamination for a manufacturing step producing cooked ground beef. Another example would be an outcome of <10% of fresh or frozen broilers contaminated with salmonellae following a slaughtering operation. To achieve the PC, Process Criteria and Product Criteria and other control measures are put in place by the food business.

Process criteria communicate the specific conditions of a particular treatment that a food must undergo at a specific step to achieve or contribute to the overall desired level of control over the relevant microbiological hazard(s), i.e. the PC. For example, the control parameter for milk pasteurization in the U.S. is 71.7 °C for 15 s (FDA 1997). This combination of temperature and time will assure the destruction of *Coxiella burnetii* (through delivering a 5D reduction) and will adequately control other non-spore-forming pathogens that are known to occur in raw milk.

Product criteria consist of chemical and/or physical parameters of a food (e.g. pH, acidulants, salt, water activity) that deliver or contribute to delivering the overall PC through limiting growth of a pathogen or contributing to its inactivation. Product criteria can also be used to assess the acceptability of a food. There is increasing recognition and acceptance that the microbial response in foods is dependent on the composition and environment in the food. Consequently, measurement of pH, water activity, temperature, and gas atmosphere affords a more rapid means of judging the safety of particular foods in which those factors are the main factors determining food safety. A food could be considered acceptable, for example, if it has been determined that a certain pH (e.g., pH $\leq$ 4.6) or water activity (e.g., $a_w \leq 0.86$) ensures that the food will meet an FSO for growth of a pathogen (e.g., *C. botulinum* or *S. aureus*, respectively).

Default criteria are conservative values established to assure the safety of a process or a food. If insufficient resources are available to perform the research needed to arrive at specific process or product criteria, then default values can be applied. An example of a default value is heating for 10 min at 90 °C internal temperature to destroy non-proteolytic *C. botulinum* in extended shelf-life ready-to-eat chilled foods (ACMSF 1992). Default values have most commonly been developed by control authorities or advisory groups. These values specify the minimum criteria that must be met to ensure the production of safe food.

From the information provided in the risk-based metrics, FSO and/or PO, regulatory authorities and food operators can select appropriate control measures to achieve the intended results. To compensate for process variability and assure that articulated risk-based metrics are consistently met, industry may implement more stringent PC for the step they control.

There is increasing recognition of the importance of contamination from the environment in which ready-to-eat foods are produced. Accordingly, information is provided on methods to assess the effectiveness of prerequisite programs as control measures (see Chap. 11).

1.12 Assessment of Control of a Process

Food management systems that incorporate the above principles require some means of verification to ensure that the systems are being implemented as planned. Criteria, e.g., process, product, etc., may be established to serve as a basis for meeting a PO/FSO, and can be used to assess whether a process is under control. A process is deemed to be under control when correct procedures are being followed and established criteria are being met. The procedures that are followed for assessing and adjusting control of a process will ideally be based upon the same principles applied in selecting the control measures. Statistical process control may be necessary when validating, monitoring, and verifying control of a process. Information on the use of statistical process control charts to monitor the performance of food operations is provided in Chap. 13.

1.13 Acceptance Criteria

Acceptance criteria are statements of conditions that differentiate acceptable from unacceptable food operations. Acceptance criteria may be sensory, chemical, physical, or microbiological and should specify ancillary information, such as the number of samples to be collected, how and where the samples are collected and held prior to analysis, the analytical unit, the method of analysis, and the range of values considered acceptable. The assessment can be performed by a control authority, a customer, or even by an independent auditor hired by the food operator, each for a different purpose. Acceptance criteria are also used to assess the acceptability of individual lots or consignments of food (see Chap. 5).

1.14 Microbiological Testing and Criteria

Microbiological testing is frequently used to determine the microbiological quality and safety of foods. The results of such testing are sometimes (incorrectly) interpreted as absolute, but due to simple statistical effects and generally substantial heterogeneity in the distribution of microorganisms in food, the results should definitely not be considered as such (Jongenburger et al. 2015; Zwietering et al. 2014, 2015, 2016). Microbiological tests are used for different purposes. It is important to consider for which purpose microbiological testing is being used. The purpose determines the type of test (indicator or pathogen), the method (e.g., rapidity, accuracy, repeatability, reproducibility), the sample (e.g., raw material, manufacturing environment, line-residue, end product), the interpretation of the result, and actions to be taken (e.g., rejection of a lot, investigational sampling, readjustment of the process, rework). Table 1.3 shows some of the many different aspects of microbiological testing that are discussed in subsequent chapters.

Under certain circumstances, microbiological criteria may be established to determine the acceptability of specific production lots of food, particularly when the conditions of production are not known (see Chap. 5), or to verify meeting PO/FSO (van Schothorst et al. 2009; Zwietering et al. 2015). Amongst other considerations, microbiological criteria should specify the number of sample units to be collected, the analytical method, and the number of analytical units that should conform to the limits (CAC 2013). The composition of a lot (batch) of food product is considered in Chap. 6. Only in well-mixed liquid foods does the distribution of microorganisms approximate homogeneity. If the distribution of microorganisms differs greatly from log-normal, the sensitivity of attributes plans is affected (see Chap. 7). The number of microbiological tests applied routinely to a lot of food product at port-of-entry is rarely adequate, in a statistical sense, to detect low levels of defectives (e.g., salmonellae in dried milk or dried egg). Moreover, random sampling is often not possible, which influences the statis-

Table 1.3 Examples of microbiological testing in food safety management

Type of testing	Purpose	User	Sample type	Sampling plan	Microbes
Acceptance	Lot inspection	Government	Endproducts	Attributes	Pathogens
Acceptance	Verification, Lots/batches of known history	Government, Industry	Endproducts, Raw materials	Attributes Attributes	Pathogens Pathogens, Indicators
Monitoring, checking	CCPs, Lines	Industry	Line samples	Variables attributes	Indicators, Pathogens
Environmental sampling	Line, environments	Industry	Residues, dust, water	Targeted, to find source of contamination	Indicators, Pathogens
Verification	HACCP	Industry	Endproducts	Attributes,	Pathogens, indicators
Surveillance	Compliance	Governments	Products in commerce	Attributes, (n = 1~5)	Pathogens
Investigation	Food chain	Governments, Industry	All types of samples	Investigational, rarely statistically based	Pathogens

tical interpretation of the results. Greater reliability can be attained by acquiring food and/or ingredients from suppliers known to have HACCP and prerequisite programs in place and with a record of trouble-free production. If a lot is sampled, the stringency of the sampling plan should reflect risk to consumers (see Chap. 8). In some situations, it may be appropriate to change the degree of sampling by applying tightened or reduced sampling plans (see Chap. 11).

When samples are collected for microbiological analysis, the method of collection and handling the samples is very important. Otherwise, the analytical results may have no bearing on the acceptability of the food. These factors are briefly summarized in Chap. 12. Examples of how these statistical principles impact the sensitivity and reliability of microbiological methods is discussed in Chap. 10.

Chapters 14, 15, 16, 17, 18 and 19 provide six examples to illustrate how the principles can be applied. Each example discusses a different aspect of control that may be necessary in a food safety management system.

1.15 Summary

The purpose of this book is to introduce the reader to a structured, more holistic approach to managing food safety. The uses and limitations of testing for control of microbiological hazards are discussed. In addition, lot acceptance testing within such safety management systems is discussed with reference to its strengths and weaknesses. The text describes the use of existing Codex documents to develop stronger, more reliable food safety management systems. Application of Codex documents in a logical sequence, together with POs/FSOs, can provide the basis for addressing issues of equivalency, levels of protection, and non-tariff trade barriers.

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Chapter 2
Evaluating Risks and Establishing Food Safety Objectives and Performance Objectives

2.1 Introduction

Societies charge public institutions and organizations with defining the "level of protection" regarding risks in daily life that should be achieved to assure the health and safety of the public. In the case of food safety, this responsibility usually resides with competent authorities that have been given this mandate by national or local legislation. Industry is responsible for assuring the safety of the products that they put onto the market or that they sell to the public. Within the context of their specific responsibilities, government and industry function as risk managers and share the common goal of ensuring that consumers can enjoy safe and wholesome foods.

Competent authorities may use different approaches when responding to an emerging food safety concern or when seeking to enhance current levels of food safety proactively. Their choice of actions to minimize risk to consumers depends on the circumstances and urgency of the situation. This flexibility is necessary because the factors surrounding concerns related to food safety vary (e.g., nature of the hazard, population affected, severity of the disease, frequency of occurrence, potential for wider dissemination of the disease agent). It is neither possible nor desirable to prescribe specific steps for control authorities to follow when responding to food safety concerns. However, some general guidelines can be given.

In addressing a food safety concern, whether wishing to reduce a current concern or to bring an increasing risk under control, risk managers must evaluate whether the situation is under sufficient control or there are sound reasons for concern. In many situations concerns are raised that, upon closer examination, are already adequately controlled by existing control measures or which do not constitute a public health issue. In the latter instance, a rapid decision must be taken to avoid wasting time and money on issues that have little impact on public health.

As new food safety concerns are recognized, some understanding of the nature and properties of the hazard, and how it leads to foodborne illness, is essential for control. Risk managers in government and industry are obliged to consider the frequency or concentration of the hazard that would be acceptable in foods and not cause illness when the food is handled and prepared as expected. Food safety managers have depended on epidemiologic studies and historical product/processing data to identify problems and determine their cause. This information then forms the basis for control options that could be applied to prevent, minimize or reduce the hazard.

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A formal process has seldom been applied to determine what a society or country would consider as an appropriate level of consumer protection in regard to a foodborne microbiological hazard. Yet, governmental risk managers have such goals in mind when developing and implementing policies and strategies for the control of microbiological hazards. There is a long history of implicitly or intuitively selecting public health protection options that provide the basis for the robust food safety management systems that currently exist.

This level of protection may not be explicitly expressed, but may be estimated from data on incidences of domestically occurring illnesses. Taking foodborne listeriosis as an example, the estimated prevalence reported for a number of countries ranged from 0.1 to 1.3 cases per 100,000 about a decade ago (Table 2.1). According to more recent data from Europe (EDCD 2013), the overall case rate for listeriosis was 0.33 per 100,000 in 2010, with highest rates reported by Finland (1.33 per 100,000 population), Denmark (1.12 per 100,000) and Sweden (0.67 per 100,000 population), while other Member States recorded rates below 0.6 per 100,000 population. US CDC presented comprehensive estimates of foodborne illnesses in 2011, in which the incidence for listeriosis was estimated as 0.53 (0.19–1.06) per 100,000 (Scallan et al. 2011). Under the auspices of the World Health Organization, the Foodborne Disease Burden Epidemiology Reference Group (FERG) conducted foodborne disease burden studies since 2007. Based on systematic reviews into the literatures and meta-analysis studies, FERG reported estimates of listeriosis cases at the global level in the year 2010 to have been 23,150 (95% credible interval 6061–91,247), 5463 (1401–21,497) deaths, and 172,823 (44,079–676,465) Disability-Adjusted-Life-Years or DALYs (Maertens de Noordhout et al. 2014). These case numbers can be converted to an estimated 2014 global incidence rate of 0.31 (0.08–1.23) per 100,000, assuming that the global population in 2014 was 7.4 billion.

Table 2.1 Reported incidence of listeriosis in selected countries

Panel	Nation	Incidence estimate (cases/100,000/year)	Period	Comment
A[a]	Australia	0.18–0.39	1991–2000	
	Canada	0.1–0.2	1990–1999	
		0.17–0.45	1987–1994	
	Denmark	0.48/0.64	1991/1992	
		0.75–0.88	1996–1998	
	Germany	0.34	Pre −1984	
		0.25	Late 1990s	
	France	0.68/1.30	1991/1992	
		0.38/0.67	1995/1996	
	Italy	0.35	1991/1992	
	Netherlands	0.13–0.19	1996–1999	
	New Zealand	0.4/0.61	1991/1992	
	Sweden	0.42	1990s	
	UK	0.14–0.23	1984–1996	Excludes outbreaks 1987–89
		0.40–0.46	1987–1989	Includes outbreaks 1987–89
	USA	0.46	1983–1992	Active reporting
		0.14	1983–1992	Passive reporting
B	USA[b]	0.53 (0.19–1.06)	2000–2008	Active surveillance; estimated US population of 299 million in 2006
	World[c]	0.31 (0.08–1.23)	1990–2012	Systematic review and meta-analysis; estimated global population of 7.41 billion in 2014

[a]Data from Ross et al. (2000)
[b]Data from USA Scallan et al. (2011)
[c]Data from Maertens de Noordhout et al. (2014)

Generally, listeriosis cases are considered to be sporadic and not associated with identified outbreaks. Although foods are recognized as the primary source of listeriosis, little is known about factors leading to sporadic cases or how they may be reduced. Most countries have therefore, albeit perhaps not deliberately, set protection at current levels and respond to unusual increases above the country's baseline. This does not mean that future reductions in the incidence of listeriosis cannot be a goal of a country's food safety enhancement program. As the factors causing sporadic cases become clearer, food safety policies can be modified to reduce the incidence of listeriosis and, thereby, achieve a higher level of protection. The availability of Whole Genome Sequencing (WGS) techniques has led to an increasing portion of sporadic cases are being redefined as geographically and temporally diffuse outbreaks.

Knowledge of the true level of protection in a society depends on the disease surveillance system. Not all countries have detailed epidemiologic data describing the current situation for every foodborne pathogen; however, some level of surveillance is in place in most countries and can be put to good use to prioritize risks posed by foodborne hazards (Gkogka et al. 2011). Moreover, analysis of a particular food production system allows identification of the hazards and factors either increasing or controlling a particular risk. Ideally, integration and application of epidemiologic data from various appropriate data systems would inform the evaluation of food safety strategies to allow proper modification of food safety programs and to determine equivalency in health protection between alternative food safety strategies (ICMSF 2006a).

Understanding of the current level of protection and setting future goals requires evaluating public health risks associated with the concentration and/or frequency of particular hazards in foods or categories of foods. This evaluation may be done in a number of different ways, depending on the issue, the scientific insights available (or key data lacking) as well as the extent that evaluation approaches can be agreed between stakeholders. In practice, the evaluation ranges from a simple qualitative estimation of risk to a quantitative risk assessment (see Sects. 2.4 and 2.7).

The current or future public health status relating to food safety may be expressed in terms of the level of "risk" to human health, i.e., either the current level of risk or a future level of risk. The latter case applies when governments or public health bodies set public health goals to inspire action to improve the current public health status and reduce the prevailing disease burden. Examples of such future public health goals are the Healthy People 2010 and 2020 target objectives articulated by policy makers in the USA (FDA/FSIS 2001, 2010) and the target set by the UK Food Standards Agency to reduce the incidence of foodborne disease by 20% by April 2006 (FSA 2000).

The World Trade Organization's Sanitary and Phytosanitary (SPS) Agreement (SPS) formally defined the "Appropriate Level Of (sanitary or phyto-sanitary) Protection (ALOP)" as: "the level of protection deemed appropriate by the Member (country) establishing a sanitary or phyto-sanitary measure to protect human, animal or plant life or health within its territory" (WTO/SPS 1994). Under the umbrella of WTO, the term ALOP is an expression that has legal weight and should be interpreted as referring solely to the level of protection that is currently achieved in a particular country (FAO/WHO 2006). Although no country has formally published ALOP values, examples of "default" ALOPs have been suggested (EFSA 2007), i.e., the incidence of *Salmonella* in Finland and Sweden at the time they joined the European Union as well as the use of the background level of cryptosporidiosis in the USA as a basis for establishing levels of treatment for drinking water. The SPS Agreement recognizes that "many members refer to this concept as the acceptable level of risk" when considering ALOP.

The Commission prefers the term "tolerable level of risk" (TLR) instead of "acceptable level of risk" (ICMSF 2002), because risks related to the consumption of food are seldom "accepted", but at best "tolerated" in lieu of various other risk priorities to be managed by governments and of continuous improvement efforts of governments and industry. Reflecting on the ALOP term, the Commission feels that using the word "appropriate" could be interpreted as a target level of protection, i.e., a level

to strive for as an endpoint, which would loose the ambition of continuous improvement underlying many public and private food safety management policies. The following phrase covers what the Commission considers the *tolerable level of risk* (TLR): "risk that society regards as tolerable in the context of, and in comparison with, other relevant risks in everyday life". The TLR is established following consideration of public health impact, technological feasibility, economic implications, etc. Like the ALOP, the TLR can be expressed in a number of different ways, for example, the number of illnesses occurring per annum due to a certain microbial hazard per 100,000 population in a country. A hypothetical example could be 0.5 cases of listeriosis per 100,000 population per year.

Although deciding on a TLR is a societal matter, sound scientific principles should underline the evaluation of risk and inform public health decision-makers. Although the dimensions and expressions of ALOP and TLR are similar, TLR is a more flexible concept as it can both relate to a current situation of public health protection being achieved in a country as well as a goal for future improvement in the level of protection.

Food operators cannot directly use a public health goal or level of protection to establish the conditions of food processing such that the necessary level of control is achieved in line with the TLR/ALOP through measures that eliminate, prevent, or reduce microbiological hazards that may contribute to the incidence of a disease. In this book and before (ICMSF 1998a, 2002), the concepts of *Food Safety Objective* (FSO) and *Performance Objective* (PO) are discussed as risk-based metrics that allow government risk managers to effectively communicate precise food safety goals to industry and trade partners. In this update of ICMSF Book 7 (ICMSF 2002), the original concept of FSO proposed by the Commission in 2002 has been aligned to that of Codex Alimentarius (CAC 2007a, 2013a), keeping to FSO for the acceptable level of a hazard at the point of consumption and using the term PO to express such levels at earlier points in the food supply chain.

FSO and PO are stated by Codex Alimentarius to signify a maximum frequency of a particular microbial hazard, a concentration or a combination of both that is considered to be tolerable for consumer protection in a particular food product or category of foods. In other words, at the point that they are established, these risk-based metrics make explicit to the industry what the upper level of a hazard is that can be tolerated in the food such that ultimately the risk at consumption is in line with the ALOP. As such, industry can then establish the adequate food safety management system at the point they are responsible to effectively control the hazard concerned to the tolerable level (i.e., the PO). Recent peer-reviewed literature has seen a number of studies interrelating ALOP and FSO and/or establishing control measures on the basis of FSO/PO (Crouch et al. 2009; Membré et al. 2007; Rieu et al. 2007; Sosa et al. 2011; Gkogka et al. 2013; Mataragas et al. 2015). While FSO and PO are newly articulated outcome targets for food safety management, verification of whether these targets are being met can be assessed using the existing food safety metrics such as microbiological criteria (MC). In association with the revised Codex guidelines on the application of MC (CAC 2013b), the Commission contributed to a working group paper in which several hypothetical studies illustrate how MC can be established to operationalize a PO or FSO (Zwietering et al. 2015).

ICMSF would propose that the concepts of FSO/PO do not only apply to pathogens, but also to their toxins or other harmful metabolites. However, the concepts do not apply to microorganisms that have no impact on the health of consumers, e.g., utility microorganisms and indicator microorganisms. In the following the current chapter, the concepts are discussed mostly in the context of pathogenic microorganisms, however, Chap. 14 provides an example of how these concepts can be used in the context of aflatoxins in peanuts.

Depending on the urgency of the situation, the availability of the necessary resources, the complexity of the hazard, and data availability and gaps, governmental and industry risk managers will need to decide on the value of setting an FSO/PO as a risk-management option, as well as on the best approach to derive values for these metrics. In principle, values for FSO/PO can be derived by advice from a few specialists, by larger expert panels, or with the aid of a quantitative risk assessment.

The FSO/PO may be based on a realistic estimate of the risk but can also, when short of time and/or knowledge, be based on a detailed examination of the frequency and/or concentration of a hazard that is expected to keep the situation under control.

The Commission considers that the establishment of meaningful FSO or PO values for particular hazard/food combinations or intervention steps along a food supply chain will typically require quantitative, risk-based approaches. Also, following the principle concept, the FSO should be derived from an articulated public health target, such as the ALOP or TLR. Because of their link with public health protection policies, FSOs can only be set by competent authorities. By setting an FSO, competent authorities articulate a risk-based limit that should be achieved operationally within the food supply chain, while providing flexibility for different production, manufacturing, distribution, marketing, and preparation approaches. Notably, an FSO could be derived from the performance of concurrent food safety management, articulating this performance to the industry or to trading partners in the language of a risk-based metric, where the public health policy is to maintain the risk at the "status quo" level. Authorities can also set advisory POs at particular points in the food chain to provide further guidance on risk-based limit to be achieved for those industries for which they consider it appropriate. However, it is expected that food business operators may wish to derive PO values for the point in the food supply chain that they are responsible for such that they ultimately align their food safety management systems such as to meet the FSO articulated by government (CAC 2007a).

Whereas a quantitative risk assessment compiled on the basis of a thorough analysis of public health data, including epidemiological surveys, knowledge of the impact of hazards on consumers, data on industry's operations and ultimate exposure of consumers to particular hazards arguably provides for the most comprehensive basis to establish values for FSOs (as well as for ALOP or TLR values), FSO values can also be derived from quantitative insight into the dose-response relationship between consumer exposure to different levels of a particular hazard and the consumer response in terms of illness. As noted above, such a hazard characterization relationship is best part of a risk assessment, but if such a curve is available for a given hazard and deemed appropriate to use for the population/situation at hand, it can be a helpful basis to relate the FSO to the ALOP even without developing a more complete quantitative risk assessment.

A PO can be derived from an FSO derived by a competent authority from a stated ALOP, or directly from the ALOP without explicitly articulating an FSO, on the basis of a quantitative risk assessment developed for a specific pathogen in a particular food for/by a competent authority or by an international intergovernmental organization with appropriate competencies. Food business operators can derive a PO from an FSO articulated by government or on the basis of a (usually quantitative) evaluation of a hazard in the part of the food supply chain for which they are responsible. In the latter situation, the PO may not be related to FSO or ALOP values set by government, but the concept itself may still be of value for coordinating food safety management across the food supply chain. For the industry, thus, the PO is the primary means for establishing the level of control needed at a specified step in the food supply chain and for communicating this to other stakeholders in and along this food supply chain. To achieve a PO, the food business operator will have to establish a food safety management system at the step in the relevant food supply chain that essentially converts the hazard level at the start of the step to the PO level at the end of the step. The metric that relates to the required conversion of the hazard level is the Performance Criterion (PC) as discussed in Chap. 1. How ALOP, FSO and PO relate to PC, MC and other metrics has been agreed on at Codex level (CAC 2007a).

It may not always necessary to articulate an FSO in relation to an ALOP or TLR, for instance when a microbiological risk assessment can relate the tolerable level of risk at the population level to one or more suitable POs along a particular food supply chain. An example would be a ready-to-eat food where the levels of a pathogenic microorganism of concern remains unchanged between product manufacture and consumption. Similarly, it may not always be useful to focus on the FSO value, for example when a competent authority seeks to communicate a default or safe-haven value for the

maximum tolerable hazard level at a certain point in the food supply chain for regulatory enforcement in the form of a PO. Enforcement at the consumption stage through articulating an FSO and assessing compliance to it would not be practical.

It is expected that, in practice, POs rather than FSOs will most often serve the purpose of making explicit the required stringency for control of a hazard. For instance, governments may articulate a PO as their expectation of what needs to be achieved by food safety management system at a particular stage in the supply chain, especially where they have or consider having enforcement related performance metrics such as a MC. Along the food supply chain, obviously, the PO concept may be a useful metric to express what level of hazard control is to be delivered as the outcome of a step in the food supply chain, which represents an input to a subsequent step, such that the material going into the subsequent step is suitable for instance for the product and process design of the food being manufactured. Through the language and the concepts of FSO and PO the various food business operators that control separate steps in the food supply chain continuum can coordinate and integrate their hazard controls in order to meet an FSO/ALOP, when articulated, or to ensure that other (quantitative) benchmarks for food safety (i.e. standards, guidelines, specifications) are being met in accordance to the food product and its intended use.

There are several notable differences between the new risk-based metrics FSO/PO and the concept of the MC that was launched in the 1980s (NAS 1985; ICMSF 1986) and adopted by Codex (CAC 2013b). These differences are shown in more detail elsewhere (see Chap. 5, Table 5.1). Essentially, FSOs and POs can help to design the required stringency of the control of food operations (Zwietering et al. 2015), but are not intended for the verification of product/process control or for determination of lot acceptance, which instead rely on metrics such as MCs (CAC 2013b; Caipo et al. 2015). FSOs/POs are also useful when comparing the safety goals of different countries or trade partners and can assist in determining equivalence of seemingly different control measures used for health protection.

Below, the concepts of FSO/PO are introduced as tools to express and communicate in practical terms the desired level of consumer protection. The following sections also provide information on some of the additional tools that have been used to characterize the public health situation.

2.2 Managing Tolerable Level of Risk

The food chain from primary production, through harvesting, processing, marketing, distribution to preparation for consumption is complex. Hazards may enter along that entire chain, starting with the source of the food and ending with its final preparation. In particular, effective control measures do not exist for the many pathogens that occur on raw agricultural commodities and seafood. At best, it is possible to reduce, but not prevent or eliminate their presence from these foods and still provide them to consumers in a raw, unprocessed state. Eliminating foodborne hazards is further complicated by the fact that controlling one hazard may increase the potential for other hazards or other adverse consequences. Thus, food control programs are oriented toward ensuring that foods are as free as practicably possible from hazards through appropriate hazard management.

For a number of foodborne diseases, the TLR is effectively the absence of disease (e.g., <0.1 case per 100,000), typically where the successful implementation of GMP/GHP and HACCP procedures and other sanitary measures over time has managed to completely control the pathogen and effectively eradicate it from the food chain, as has occurred with foodborne brucellosis in certain regions such as Europe (ECDC 2013).

Food safety management is similar to managing other risks in human life. For a range of hazards, society balances the risks and benefits and, although rarely stated publicly, accepts that a certain risk has to be tolerated. For example, the risk of injury while driving a car can be reduced by designing

safer vehicles, regulating traffic, setting speed controls, wearing seat belts and driving defensively. Despite all such efforts, society has come to accept that a certain number of accidents will occur. However, many consumers expect food to be safe and have little or no tolerance for purchasing food that may cause illness.

Managing tolerable risk implies balancing public health considerations with other factors such as economic costs, public acceptability, etc. Based on epidemiologic data and an evaluation of risk, risk managers in government should decide whether a particular risk is so small that no further action needs to be taken, that this risk needs to be contained at the actual level (e.g., because present systems already in place are adequate), or that the risk needs to be reduced to a tolerable level. When deciding that microbiological risks need to be reduced, governmental risk managers should consider the various risk management options (CAC 2007a), which include communicating to industry the need to more tightly control hazards in the food chain and the level of stringency for the control that is expected by the risk managers. However, it must be realized that there will be a point at which further reductions in risks associated with specific foods may have additional "costs" that society is not willing to bear. Therefore, there is a need to balance the benefits of risk reduction with the costs incurred.

Various tools for cost-benefit analysis have been developed to estimate the economic impact of decisions of this nature. A key component of any estimate of economic costs is the impact that foodborne disease has in terms of medical costs, lost productivity, loss of consumer confidence, etc. As an example, it was estimated that between 3.3 and 12.3 million cases of foodborne illness occurred each year in the USA due to 6 bacteria (*Campylobacter jejuni / coli*, *Clostridium perfringens*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica*, *Staphylococcus aureus*) and one parasite (*Toxoplasma gondii*). The medical costs and losses of productivity, in US dollars in 1995, associated with these 7 pathogens were estimated to be between $5.6 and $9.4 billion (Buzby and Roberts 1997). A more recent study considered the latest surveillance data from the USA (Scallan et al. 2011), and estimated the public health burden of 14 pathogens to be over $14 billion in terms of the monetary losses associated with illness, with 90% due to only five pathogens: *Salmonella enterica* (non-typhoidal), *Campylobacter* spp., *Listeria monocytogenes*, *Toxoplasma gondii*, and norovirus (Batz et al. 2012; Hoffmann et al. 2012; Tam et al. 2012). Long-term complications arising from acute infections were identified to constitute a substantial portion of the costs associated with *Listeria, Campylobacter* and *Toxoplasma*.

It has to be acknowledged that costs are not limited to economic concerns. As another hypothetical example, suppose that changing a traditional, religiously based food handling practice would reduce the risk of foodborne illness. While modifying these practices might provide a small reduction in risk, the cost associated with the loss of choice based on religious beliefs may be considered unacceptable and the affected population may prefer to tolerate the slightly higher risk.

Similarly, the impact that food control requirements have on the freedom of consumers to make decisions on the foods they eat must be recognized. However, there are several instances where the potential public health consequences of specific foods or food handling practices potentially are so severe that consumer choices may need to be restricted where society will not communally tolerate the risks that some consumers as individuals are willing to take. For example, a number of countries prohibit the commercial sale of non-pasteurized milk for direct consumption due to concerns about the possible presence of a number of different hazards posing consumer risks. Other examples are bans existing in a number of countries on the sale of uneviscerated salted fish, due to the potential risk of growth and toxin production by *C. botulinum*, or on import of fish from the order *Tetraodontiae* (e.g., puffer fish), due to potential risk of tetrodotoxin poisoning.

In these examples, the products inherently exceed the risk tolerated in the countries affected and, other than prohibition, there are no viable control measures. The foods mentioned have a long history of public health problems and risk managers in some governments have concluded the risks were not tolerable within the context of their society. Nevertheless, it is equally possible that another society values these foods highly and would be willing to tolerate the associated public health consequences.

Another cost that must be considered is when risk management measures taken to reduce the risk of one hazard are likely to increase the risk associated with another hazard or will have other adverse (health) effects, e.g., on nutritional properties. A pertinent microbiological example is the use of chlorine for the treatment of water for drinking and food processing. While chlorination reduces risks associated with water-borne microbial diseases, there is a concentration-dependent risk that results from formation of organo-chlorine compounds (FAO/WHO 2009b). A hypothetical example would be a ban on pasteurized milk because it has been associated with a notable risk for listeriosis at the population level (note that the risk per serving is low) (FAO/WHO 2004), but which is a very important source of calcium and other nutrients that are essential for health. These situations require risk management decisions that balance two competing issues such that the benefits outweigh the risk.

Most societies consider consumer protection a moral responsibility, deserving high priority. With increased reporting of foodborne illness by the media, consumers have become more aware of the frequency and public health impact. This increased awareness has led to greater pressure on governments and industry to make changes that will further reduce risk. This could be reflected in the future levels of risk that will be tolerated for various foodborne microbiological hazards.

A decision not to take an action also has its costs in relation to all of the categories identified above.

The net result of balancing the various risks and benefits is a decision on which actions have to be taken. To implement the action(s), an objective must be defined. This objective may be expressed for instance in terms of a (maximum) tolerable level of a hazard in a food (i.e., PO or FSO) or as a public health goal regarding risk in the population (i.e. ALOP or TLR). As noted before, expressing population level goals in terms of risk (e.g., cases per 100,000 population per annum) does not provide the explicit guidance required by the industry or inspection and control authorities in the government. Likely, the most effective means to ensure that the actions taken will be achieving a goal is to express it in terms of the level of hazard. This is a key rationale underlying the introduction of the new risk-based metrics, FSO and PO, and their adoption by Codex Alimentarius.

2.3 Importance of Epidemiologic Data

Accurate knowledge of disease incidence and severity is critical for competent national authorities for use in shaping risk-based public policy, including setting ALOP or TLR and selecting appropriate management actions to reduce the overall public health impact, including FSO and/or PO or other options.

Levels of risk to consumers in a country are generally articulated in relation to the mortality or morbidity of a disease, expressed as a number of cases (morbidity) or deaths (mortality) for a certain size of population per period of time. For many reasons the “true” incidence of foodborne disease is not known. At best, reasonable estimates can be developed for particular diseases because the impact on the consumer is profound and the characteristics of the diseases are sufficiently evident.

At the basis of any articulation of risk, e.g., current risk, tolerable risk or future goals to mitigate risks, lies the collection, synthesis and analysis of data from a number of epidemiologic data sources that together signal the emergence or existence of food safety problems, characterize them and allow assessment of the effectiveness of control measures at the levels of the food supply and the human population (Table 2.2). The following datasets are considered to be within the scope of epidemiologic data (ICMSF 2006a):

- data derived from vital records, registries, and surveillance of clinical diseases in humans, plants, and animals
- data from epidemiologic investigations of outbreaks and other special public health studies
- data from laboratory-based surveillance of pathogens isolated from humans, plants, animals, and food processing environments

Table 2.2 Surveillance data needs within the food chain for adequate evaluation of prevention effectiveness (ICSMF 2006a, b)

Pre-harvest	Country-level surveillance of food animal and plant diseases and the occurrence of pathogenic agents transmissible to humans through food should be able to rank commodities by frequency of contamination and determine subtype/fingerprint of hazards by animal or plant of origin, geographic origin, production practices and conditions, and season.
Harvest, processing, distribution	Country-level microbial surveillance and monitoring programs should be able to rank specific foods by frequency of contamination, and determine subtype/fingerprint of hazard by food, process, step in process, geographic origin, and season.
Retail food service, retail sale and home preparation	Country-level microbial surveillance and environmental sanitation and behavioral risk factor surveys should be able to: (1) rank specific foods at retail by frequency of contamination, (2) associate environmental antecedents (practices, processes, behaviors, and equipment) at retail with product contamination, (3) provide a subtype/fingerprint catalog of hazard by food, practice, process, behavior, geographic origin, and season, and (4) characterize consumer-induced risk factors for foodborne disease (e.g., host factors, food choices, and home food handling practices).
Public health surveillance	Country-level public health surveillance systems should be able to estimate frequency of adverse health events from hazards that are frequently foodborne and track the occurrence of infections that can be transmitted from infected persons to consumers through contamination of food. Routine epidemiologic studies of sporadic foodborne disease should be able to allocate the relative proportion of major hazards attributable to specific foods, at least within broad food categories. Country-level outbreak investigations and surveillance should be able to identify food sources of epidemic disease and allocate the relative proportion of major hazards attributable to specific foods, processes, practices, behaviors, and host characteristics for epidemic disease. Enhanced attribution of foodborne illness to specific foods, processes, and behaviors is essential for accurate assessment of the public health effectiveness of management strategies.

- monitoring data derived from regulatory and non-regulatory sampling of foods, including microbial food testing
- environmental health data on practices and procedures of food workers
- data from behavioral surveillance of consumer habits and practices.

Information from various sources is very important to compile, analyze and understand in terms of the burden of illness on a population and to assess the effect of policies instituted to thwart foodborne disease. Several approaches are currently used to monitor and report the incidence of foodborne diseases:

- passive notification systems
- active surveillance systems
- case control studies
- outbreak investigations
- sentinel studies

None of these systems yield all the data necessary for a quantitative risk assessment and some (e.g., passive notification systems) often fail to identify food as a source. Passive notification systems follow trends in disease and can be useful for measuring the impact of changes in technology, preventive measures and regulatory policies. For example, Fig. 2.1 shows the reported incidence of salmonellosis and shigellosis in the U.S.A. from 1980 through to 2013 (CDC 2015a). The data were developed through reports from local sources in each state and then submitted from the state to the Centers for Disease Control and Prevention. In addition, physicians have been required to report particular "notifiable" diseases. This mandatory requirement can strengthen the accuracy of the data but many cases remain unreported. Similarly, reports from laboratories can identify trends for instance in non-typhoidal *Salmonella* cases and identify for public health officials changing risks to the population (Fig. 2.2).

Another approach to collecting data on the incidence of disease is through active surveillance systems such as EnterNet or FoodNet (MMWR 2000, 2013). EnterNet has been established to determine

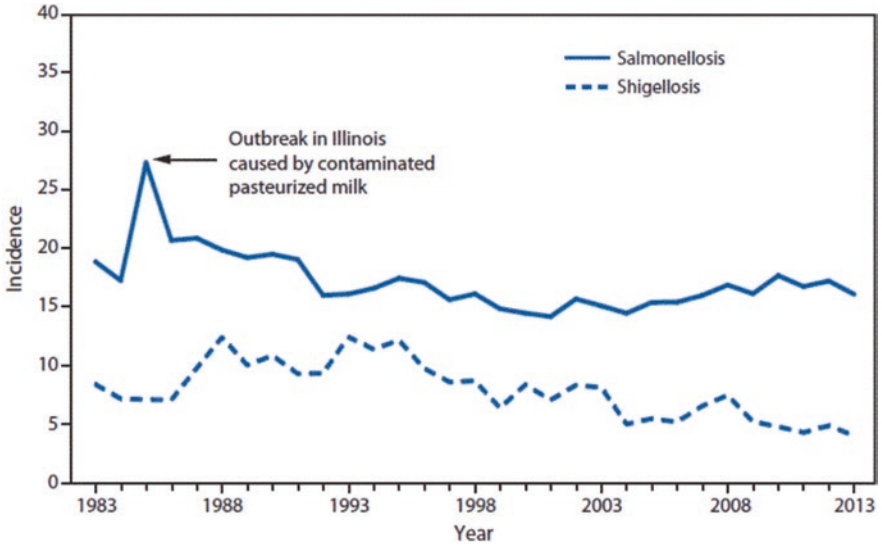

Fig. 2.1 Trend in the incidence per 100,000 population of salmonellosis and shigellosis cases in the United States from 1980 to 2013 (CDC 2015a)

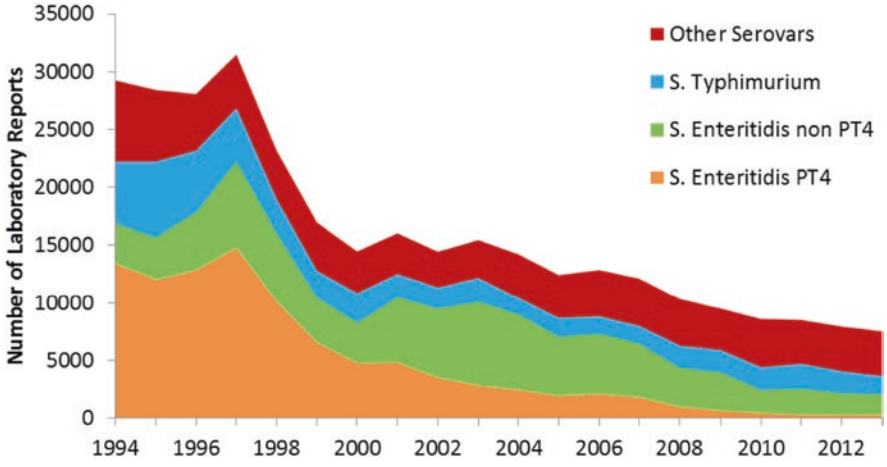

Fig. 2.2 Laboratory reports of non-typhoidal human *Salmonella* cases in the UK, 1994–2013 (DEFRA 2015)

more accurately the incidence of salmonellosis and infections caused by *E. coli* O157 in Europe. Another goal of EnterNet is to establish a system to identify outbreaks in Europe from a common food source. First established in the USA in 1996, and later duplicated and implemented in several regions around the world, the Foodborne Diseases Active Surveillance Network or, FoodNet, is an active, sentinel site program that collects weekly updates from clinicians in certain regions of the country for specific foodborne illnesses, including *Campylobacter*, *Cryptosporidium*, *Cyclospora*, *Listeria*, *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC) O157 and non-O157, *Shigella*, *Vibrio* and *Yersinia*. Isolates of selected pathogens are compared for commonality to identify outbreaks due to a common food source. FoodNet estimates the number of foodborne illnesses in the USA, monitors trends in incidence of specific foodborne illnesses over time, attributes illnesses to specific foods and settings, and disseminates

Table 2.3 Incidence per 100,000 population of culture-confirmed bacterial and laboratory-confirmed parasitic infections, and post-diarrheal hemolytic uremic syndrome (HUS), by year and pathogen, in the United States between 1996 and 2014 by the Foodborne Diseases Active Surveillance and reference incidences from the National Health Objective (NHO) for 2010 and 2020 (amended from CDC 2015b)

Pathogen/syndrome	Year								NHO 2010§	NHO 2020¶
	2000	2002	2006	2008	2010	2012	2014	2014		
Campylobacter	15.36	13.38	12.73	12.65	13.53	14.22	13.45	13.45	12.3	8.5
*Listeria**	0.33	0.25	0.28	0.26	0.27	0.26	0.24	0.24	0.24	0.2
Salmonella	14.08	16.24	14.76	16.10	17.55	16.38	15.45	15.45	6.8	11.4
Shigella	7.67	10.86	6.10	6.57	3.77	4.47	5.81	5.81	N/A†	N/A
STEC§§ O157	2.03	1.69	1.30	1.12	0.95	1.11	0.92	0.92	1.0	0.6
STEC non-O157	0.19	0.16	0.53	0.53	0.96	1.16	1.43	1.43	N/A	N/A
Vibrio	0.18	0.27	0.34	0.29	0.41	0.41	0.45	0.45	N/A	0.2
Yersinia	0.43	0.45	0.36	0.36	0.34	0.33	0.28	0.28	N/A	0.3
Cryptosporidium	1.57	1.32	1.94	2.27	2.75	2.63	2.44	2.44	N/A	N/A
Cyclospora	0.06	0.10	0.09	0.04	0.06	0.03	0.05	0.05	N/A	N/A
HUS**	2.04	2.05	2.21	1.71	1.88	1.47	–	–	N/A	0.9
Surveillance population (millions)***	**30.64**	**37.86**	**45.32**	**46.33**	**47.15**	**47.89**	**48.24**	**48.24**		

§, ¶: National Health Objective target for incidence of indicated pathogen in Healthy People 2010 and 2020, respectively

*: *Listeria* cases defined as isolation of *L. monocytogenes* from a normally sterile site or, in the setting of miscarriage or stillbirth, isolation of *L. monocytogenes* from placental or fetal tissue

†: No National Health Objective exists for the indicated pathogen

§§: Shiga toxin-producing *Escherichia coli*

¶¶: Surveillance not conducted for this pathogen in this year

**Incidence of postdiarrheal HUS in children aged <5 years; denominator is surveillance population aged <5 years

***Preliminary U.S. Census Bureau population estimates for 2013

this information. Table 2.3 summarizes surveillance data collected by FoodNet between 1996 and 2014 in terms of rates of incidents for various illnesses tracked. For the relevant illnesses, the rates assessed are compared to the stated public health goals under the Healthy People 2010 and 2020 initiatives (FDA/FSIS 2001, 2010), showing the USA's ambition for continuous improvement of public health protection.

It is difficult to compare outcomes of epidemiological surveillance between different countries or regions in the world. Surveillance systems differ and most capture only a proportion of the cases occurring in their country/region. Moreover, cases of particular diseases remain undiagnosed (aka under-ascertainment) and/or diagnosed but not reported to public health authorities (aka underreporting). Under-ascertainment and underreporting levels vary by disease and country/regions as it involves a complex mix of healthcare-seeking behavior, access to health services, availability of diagnostic tests, reporting practices by doctors and others, and the operation of the surveillance system itself. Even within a harmonized region such as the European Union, data provided by the 26 Member States on the 52 communicable diseases and health issues for which surveillance is mandatory show inconsistencies for these and other reasons (ECDC 2014).

Data on foodborne disease is also collected through case control studies by interviewing patients to learn their food consumption history and to identify food sources. In parallel, a number of individuals are selected to serve as controls. This methodology has been used to identify not only the foods that may be involved, but also risk factors that the patients may share and that may explain increased susceptibility to the disease. Case-control studies are useful for identifying pathogen-food combinations where it has been difficult to isolate the causative organism from the food source or the role of foods in diseases with long incubation times before onset of symptoms (e.g. listeriosis).

The identity of the food source and conditions leading to foodborne illness may also be determined through epidemiologic investigations of outbreaks. Unfortunately, not all outbreaks are adequately investigated or described fully in the scientific literature, particularly those that do not provide new information. Consequently, that literature is often of limited use in relation to establishing the true frequency of their occurrence and thus the risk(s) associated with the disease agent. Case control studies can also be used to help identify the source(s) of sporadic cases of foodborne illness and the factors that contribute to their frequency. Different sources may be more important in sporadic cases than in outbreaks. In the USA, outbreaks of *Campylobacter jejuni* infections in the spring and fall are typically caused by drinking raw unpasteurized milk or untreated water, whereas sporadic cases occurring in the summer appear related to touching or consuming uncooked poultry (Potter and Tauxe 1997; Tauxe 1992).

A sentinal study monitors selected health events in a group of persons representative of the whole population. Laboratory testing may be limited, e.g., to patients reporting diarrhea, or may include examination of all fecal samples for a range of pathogens. This approach, for instance, has been used to estimate the incidence of campylobacteriosis and salmonellosis in The Netherlands (Notermans and Hoogenboom-Verdegaal 1992) and England (Wheeler et al. 1999; FSA 2000).

However, much of the information collated regarding foodborne illnesses by different systems cannot be directly translated into policy since:

- not all cases are reported to health authorities, resulting in considerable uncertainty about the actual burden of illness
- often only a fraction of illnesses caused by food-related pathogens are actually foodborne because transmission can also be through the environment, direct contact with animals, or from person to person
- foodborne illnesses may vary both in incidence and severity, resulting in widely different clinical manifestations and potential likelihood of long-term sequelae.

Targeted studies in a number of countries have attempted to estimate the magnitude of underreporting. Mead et al. (1999) for instance estimated underreporting factors for different illnesses ranging from 2 for botulism and listeriosis, 20 for EHEC and shigellosis, and 38 for campylobacterioses and salmonellosis. In general, the estimates generated by such studies reflect a similar degree of magnitude in countries of similar economic development, demographics and healthcare infrastructure, but differences do exist. According to European studies similar to the one conducted in the USA, found the underreporting factors for campylobacteriosis were 7.6 (Wheeler et al. 1999) and 10.3 (Adak et al. 2002), while these studies reported underreporting factors of 3.2 and 3.9, respectively, for salmonellosis.

2.4 Evaluation of Risk

2.4.1 Introduction

When a food safety problem or the need for improvement in the food safety status is identified, it is the responsibility of the governmental risk manager to conduct a risk evaluation to determine the magnitude of the problem as well as to decide whether and what action(s) should be taken. An evaluation of risk and the documentation thereof may take many different shapes, depending on available data and resources. For instance, the risk evaluation may be performed by one or more experts, an (multifunctional) expert panel, or other experienced resources. The risk evaluation may be established in the form of a risk profile (CAC 2007a), a descriptive/qualitative risk assessment or even an in-depth quantitative risk assessment. FAO/WHO and Codex Alimentarius have issued elaborate guidance for risk managers on this in the context of the Risk Analysis framework that underlies the work of Codex Alimentarius committees and the work of the Joint Expert Meeting on Microbiological Risk Assessment (JEMRA) (CAC 1999, 2007; FAO/WHO 2000, 2002, 2003, 2008a, 2009a).

Risk is a measure of the probability and consequence of uncertain future events (Gorris and Yoe 2014), which in the area of food safety can be reflected as:

Risk = Exposure to hazard ∗ Consequence of exposure

Exposure to the hazard may be determined by the hazard concentration and prevalence in the food as well as the amount and frequency of the food consumed. There is an element of chance that exposure occurs, which needs to be accounted for when assessing risk. The consequence of exposure then is characterized by the resulting outcome of exposure for the consumer, which usually is an adverse health effect, i.e. illness, but which can vary very significantly among consumers.Effective management of microbial hazards requires identification of the hazards, assessment of the risks associated with those hazards, determination of possible options to mitigate risks and estimation of the effectiveness of the potential options to manage microbial hazards down to a tolerable level of risk. The stringency of control that the risk management option(s) of choice put on the food system should be proportional to the risk of the particular hazards to public health. This principle also underlies modern food safety management systems for food operations, such as Hazard Analysis Critical Control Point (HACCP), that are based on preventive strategies.

Historically, food control agencies and industry have relied on the judgement of one or more experts to estimate the risk and the corresponding level of control needed to manage it. Although this approach has often been successful, it can be biased or inconsistent. Further, it can be difficult to transparently communicate the underlying scientific basis and rationale for the decisions to interested parties because such evaluations have often been inadequately documented.

An improved approach is the use of structured safety assessments, typically including broader scientific expertise and more formal consideration of available data and information. This approach has been widely used in recent years where expert panels have been called to address various food safety issues, for instance psychrotrophic *Clostridium botulinum* in refrigerated foods with extended shelf life (ACMSF 1992), *Listeria* in ready-to-eat foods (Anonymous 1999, ICMSF 1994), *Mycobacterium avium* subspecies *paratuberculosis* in milk (NACMCF 2010), and *Salmonella* and norovirus in tomatoes (EFSA 2010).

Over the last decade, quantitative microbial risk assessment approaches have been initiated and more broadly adopted to systematically evaluate the impact of various factors such as the host, the pathogen, and the type of food (liquid, solid, fat) that contribute to the risk associated with a foodborne microbiological hazard.

Risk assessment is mentioned as a tool in the WTO/SPS agreement to assure that international trade in food is not hampered by unjustified safety requirements. This has led to an international harmonization of the concept and its practical implications by the Codex Alimentarius Commission (CAC 1999), although there is, as yet, no general (or international) agreement on when a quantitative risk assessment is necessary or which statistical/mathematical approaches are appropriate. Under the auspices of FAO and WHO, JEMRA has provided a range of international microbiological risk assessments and guidance documents that are made available on dedicated websites of FAO and WHO.

There is a range of recent accessible resources on the principles of risk analysis (Moy 2014) and its component parts of risk management (Motarjemi and Moy 2014), risk assessment (Gorris and Yoe 2014; Ross 2014) and risk communication (Motarjemi and Ross 2014) with regard to microbiological hazards.

The first step in any evaluation of risk is the identification of a food safety problem from one or more sources, such as accumulated epidemiologic data, governmental or intergovernmental bodies, public health sectors, the food industry, expert opinion/scientists, NGOs and consumers. Background information is generally assembled by a governmental risk manager, possibly with input of subject matter experts from public or private organizations, using readily available data and information to sufficiently describe the problem. In the Codex context, it has been advocated that the identification of a food safety problem is articulated through the development of a risk profile. Certain regions and countries around the globe have compiled risk profiles on pathogen-food combinations. For examples of risk profiles see, for instance, foodrisk.org/rm/riskprofile.

On the basis of the information available on a particular food safety problem and the urgency considered for its management, governmental risk managers must decide whether to direct risk assessors to initiate further studies and bring together a more elaborate qualitative or quantitative evaluation to obtain the information necessary for risk management decisions, e.g., understanding of the level of risk to (sub)populations of consumers, factors contributing to risk, possible risk mitigation options. The purpose of a risk assessment is to enable a risk manager to make informed decisions. What form of risk assessment should be adequate for the risk manager to base their decision(s), and will depend on the food safety problem, factors such as the data/information already available or missing, and the feasibility to close key gaps in data/information with available resources in a timely manner. It is important that there is a common understanding of the problem between the risk managers and the risk assessors when a risk assessment is commissioned by the risk manager. This understanding should then include the risk assessment format to be taken (qualitative or quantitative assessment, deterministic or probabilistic), the available resources, time constraints, and the desired form of the output, e.g., a risk estimate (probability of disease per exposure or per year to the population or a subpopulation) and/or suggested control measures. In the Codex context, for microbiological issues the Codex Committee for Food Hygiene (CCFH) acts as the risk manager and the Joint Expert meeting for Microbial Risk Assessment acts as the risk assessor, CCFH establishes a specific set of questions (i.e. FAO/WHO 2004) or a "terms of reference for scientific advice" (FAO/WHO 2008b) for JEMRA as the basis for the elaboration of an appropriate risk assessment.

Circumstances may lead a risk manager to seek the information needed for decision making first through a mainly qualitative evaluation (e.g., expert panel), commissioning a more detailed quantitative assessment as necessary. The best course of action likely is a case-by-case decision. Over the years, various organizations have shared learnings and resources. ILSI Europe for instances gathered experiences on the practical utility and validity of microbiological risk assessment for risk management (Lammerding 2007) and produced an overview of tools available for MRAs (Bassett et al. 2012). Several governments have published guidelines regarding MRA (FSANZ 2005; USDA/FSIS/EPA 2012).

Basic aspects of both qualitative and quantitative risk assessments will be discussed in the following text. The use of expert panels as a form of qualitative risk assessment will be discussed first followed by quantitative risk assessment (see Sect. 2.7).

2.4.2 Use of Expert Panels

Control authorities, and others, have found expert panels to be an effective means to assemble information, interpret its content and develop recommendations in a relatively short period of time. Expert panels have been used extensively by governments and international bodies to address concerns about the safety related to a particular hazard-food combination or to provide other relevant food safety advice. Examples are the JEMRA consultations previously referred to, the various panels set-up under the European Food Safety Authority, such as the Panel on Biological Hazards (BIOHAZ), and the Advisory Committee on the Microbiological Safety of Food (ACMSF) and the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) that informs multiple agencies with the US government. In addition, expert panels have been used by industry to consider the factors leading to foodborne disease and develop recommendations for their control (e.g., Nickelson et al. 1996; Lammerding et al. 1999). Assessments by expert panels may be appropriate if advice is needed quickly to manage a newly recognized concern, if resources and/or data for a (quantitative) risk assessment are limited, or where there are few management options. Such panels are possibly also called upon when, in particular, epidemiological evidence indicates that a hazard is not under control and there is need for increased consumer protection. Furthermore, concerns may be raised following

change in food habits, food processing technologies, and food packaging or distribution systems. Such concerns must be evaluated and, if reasonable and supported by scientific evidence, appropriate management of the risk must be undertaken.

In practice, risk managers will call upon people with expertise on the particular pathogen and/or food in question and preferably contributing expertise from different scientific or technical areas. Panels therefore may consist for instance of epidemiologists, public health specialists, risk analysts, food microbiologists and food technologists with knowledge about actual food processing operations, important for the evaluation. The panel will be asked to provide the best information available at that given point in time. Although the complexity of risk evaluations may vary case-by-case, panels typically go through a range of steps that, following international risk analysis frameworks such as that of Codex (CAC 1999) are referred to as hazard identification, hazard characterization, exposure assessment and risk characterization. Typically, panels would accumulate available quantitative and qualitative data on the food safety problem at hand, develop a view on the risk to a consumer population or sub-populations, identify factors that contribute to risk or could mitigate risk, review options for risk managers to mitigate risk to lower levels possible following a number of scenarios of risk characterization and risk mitigation, and establish recommendations for risk managers to consider. The panels will also identify gaps in available data or where there are major aspects of uncertainty or variability in the assessment of the panel and communicate this to the risk managers. In some instances a rough estimation of the risks associated with different likely scenarios is sufficient. One approach is to assign relative probability and impact rankings, such as negligible, low, medium, or high, to the factors used to determine likelihood of exposure and likelihood of an adverse outcome. If such a system is used, definitions and rationale for assigned rankings must be clearly described and justified to avoid misinterpretations of the information by users. An example follows in Chap. 8, where different hazards are ranked into categories dependent on the severity of the disease. The outcome from an expert panel may be to recommend to risk managers one or more measures to control a hazard or, if necessary, ban the product or process. Where appropriate, an expert panel may recommend the establishment of an FSO or PO where their work provides the necessary support that implementing such metrics could be an effective means to enhance consumer protection related to the particular hazard-food combination or the food safety situation under consideration. If there is significant uncertainty, the panels may recommend interim measures that should be taken until gaps in information and data can be addressed.

Chapters 14, 15, 16, 17, 18 and 19 are examples of risk evaluations conducted through the work of expert panels. Further discussion of the role of quantitative risk assessment in development of FSOs and POs is provided in Sect. 2.7.

2.5 Food Safety Objectives and Performance Objectives

Governments and food industries have a significant influence on the incidence of foodborne disease by controlling the frequency and extent of contamination of foodstuffs and other conditions that minimize or control foodborne diseases. Consequently, public health goals must be converted into parameters that can be controlled by food producers and monitored by government agencies. A Food Safety Objective (FSO) set by government at the point of consumption may provide such a conversion. However, as has been discussed in Chap. 1, government or industry may derive from an FSO a so-called Performance Objective (PO) at an earlier point in the food supply chain. FSOs/POs establish the stringency under which food control systems must operate by specifying the frequency or concentration of a microbiological hazard that should not be exceeded at the moment of consumption. It thereby forms the basis by which control authorities can establish standards or guidelines and assess whether an operation is in compliance and is producing safe foods, i.e. the foods will under normal conditions of commercialization and use meet the established FSO.

While FSOs and POs can obviously be set for any food hazard (e.g. carcinogens, pesticides, toxins, microorganisms), in the context of this book, only hazards of microbial origin are considered. Therefore, it will be implicit that the FSOs/POs dealt concern microbiological food safety or performance objectives. The literature on the merits of FSOs/POs as concepts has been building up over the last decade and the range of views is well worth appraising (e.g., Havelaar et al. 2004; Zwietering 2005; Rieu et al. 2007; Whiting 2011; Pitt et al. 2013; De Cesare et al. 2014; Manfreda et al. 2014; Whiting and Buchanan 2014). Laymen language as well as graphical information on these concepts can be found on the ICMSF website (ICMSF 2006b).

See Chap, 1, Sects. 1.3 and 1.10 for an introduction to current definitions and advocated use of the concepts of FSO and PO. Chap. 1, Sect. 1.11 provides an introduction to related risk metrics (i.e. PC, MC).

2.5.1 Examples of FSOs/POs

An FSO is the maximum frequency and/or concentration of a microbial hazard in a food at the moment that the food is consumer that is considered tolerable for consumer protection. Similarly, a PO signifies the tolerable level at a specific earlier point in the food chain, for instance at manufacturing or at retail. It is important to re-emphasize that the primary purpose of an FSO/PO is to translate a public health goal (i.e., a desired level of consumer protection) to measurable attributes that allow industry to set establish an adequate food safety management system at the point of the food supply chain they have responsibility for. Next to that, FSOs may allow comparison between countries related to the level of consumer protection that is expected in the context of food trade.

Although Codex has adopted the concept of FSO/PO and related risk management metrics (CAC 2013a) and established guidelines for their use in risk management (CAC 2007a), these concepts are still rather new and not yet used in food legislation. Some jurisdictions however do mention the terms in their legislation, without specific reference to pathogen-food combinations and/or values for the concepts. Therefore, only hypothetical examples of FSOs or POs can be given to illustrate the concepts.

Some examples of FSOs (all valid at consumption) are:

- The amount of staphylococcal enterotoxin in cheese must not exceed 1μg/100 g.
- The concentration of aflatoxin in peanuts destined for further processing must not exceed 15 μg/kg.
- The level of *L. monocytogenes* in ready-to-eat foods must not exceed 100 cfu/g.
- The concentration of salmonellae must be less than 1 cfu/100 kg of milk powder.

Examples of POs (valid at specific points in the food supply chain) are:

- The prevalence of *Salmonella* spp. in neck skin samples taken after the carcass chill step for raw poultry meat carcasses should be ≤10%.
- The level of pathogenic *E. coli* shall not exceed 1 cfu/10 L when fruit juice is packaged for distribution.
- At the end of manufacture, the level of *L. monocytogenes* in cold smoked salmon with a 2 week shelf-life at 4 °C should be ≤25 cfu/g assuming 0.6 log cfu growth of the pathogen during shelf-life
- Ready-to-eat foods not supporting the growth of *L. monocytogenes* must not exceed 100 cfu/g at the time they are placed on the market (i.e. at retail level).

For governments to establish such maximum hazard frequencies and/or levels requires sound quantitative insights in the hazard-food product combination at relevant points in the food supply chain.

Notably, the above examples of FSOs/POs are presented as "lines in the sand", focusing on either concentration or prevalence as a tolerable limit for a hazard. While Codex indeed defines FSOs/POs as maximum *frequencies and/or concentrations* of pathogens that are considered tolerable, various authors have argued that it is important to account for *both* frequency (prevalence) and concentration (level) of a hazard when articulating an FSO/PO (Zwietering 2005; Havelaar et al. 2004) to provide the relevant industry with proper quantitative targets to be met by implementing adequate food safety management systems.

Moreover, to make the use of risk-based metrics such as FSOs/POs useful in practice, the authority establishing the metric needs to specify the expected level of control beyond a mere "line in the sand" limit, e.g. by defining what proportion (e.g., 95%, 99%, 99.9%, etc.) of the distribution of possible concentrations must satisfy the test limit so that the FSO (or the PO set by government) is met (van Schothorst et al. 2009). In other words, the proportion of the lot that may be above the nominally 'acceptable' level, i.e. the "tolerance" for testing compliance must be specified.

To be able to do this, insight is needed in the distribution of possible contamination concentrations that is typical for the product at hand. Given the expected level of control is articulated, evidently, some units of food will exceed the values specified as the limit in the FSO/PO. Provided that the proportion of such units is within the limits expected for the distribution around the mean contamination level required to achieve the FSO/PO, it may be assumed that the food safety management system operates with the expected level of control over the hazard.

For instance, as a hypothetical example, a competent authority has set the value of a PO for *E. coli* O157 in apple juice at the end of manufacture as: absence of *E. coli* O157 in 99% of 100 ml units of apple juice. Next, the authority defines the nominally 'acceptable' level at 1% (but obviously other values could have been chosen), due to which the PO is then understood as being the 99th percentile of a cumulative frequency distribution of log concentrations. The overall guidance for the industry thus is that in the case no more that 1% of the product units exceed the PO with 99% confidence in the test, then the food safety risk management system is operating as intended. Choosing the value for PO as well as its tolerance are risk management decisions, because they clearly influence the presence of a hazard that is considered acceptable.

The decisions concerning the value(s) for frequency/concentration of a FSO/PO and the expected level of control are also key requirements to allow for deriving a Microbiological Criterion at the point of the FSO/PO that can be used to verify at the operational level, i.e. at the level of batches/lots being produced, whether the food safety management system in place operates as required (van Schothorst et al. 2009; Zwietering et al. 2014, 2015).

Whenever possible, FSOs/POs should be quantitative and verifiable. However, this does not mean that they must be verifiable by microbiological testing. For example, an FSO for low acid canned foods might be established in terms of the probability of a viable spore of *C. botulinum* being present as fewer than 0.000000000001 per can. It would be impossible to verify this metric by end product testing, but it would be verifiable by measurement of time/temperature protocols that are based on a performance criterion (see Chap. 3).

Where government has stipulated mandatory FSOs/POs, industry should validate that their food safety management system is capable of controlling the hazard of concern to the expected extent, i.e. by providing evidence that the (set of) control measures underlying the system can consistently meet the FSOs/POs at the relevant scale (CAC 2008a; Zwietering et al. 2010). In addition, during ongoing operation, industry should periodically verify that their food safety management system is functioning as intended (CAC 2013b). Control authorities may rely on inspection procedures (e.g., physical examination of manufacturing facilities, review of HACCP monitoring and verification records, analysis of samples) to verify the adequacy of food safety management systems adopted by industry in meeting set FSO/PO values. Microbiological criteria are envisaged to play an important role in making FSOs/POs operational, both from the government as well as the industry perspective (CAC 2007a; van Schothorst et al. 2009; Zwietering et al. 2010, 2014).

While FSOs and POs at first glance seem similar to microbiological criteria, they differ in several ways (see Chap. 5). FSOs/POs are not applied to individual lots or consignments and they do not specify sampling plans, number of analytical units, etc. (Zwietering et al. 2014). Most often, POs are used to define the level of control that is expected for a food operation and can be met through the implementation of GMP/GHP and HACCP systems and application of performance criteria, process/product criteria and/or acceptance criteria, whereas the FSOs provide for an outcome-oriented target for the food supply chain as a whole (see Chap. 3).

2.5.2 The Use of FSOs and POs

Through the articulation of FSOs and/or POs, as appropriate, authorities set-out to communicate clearly to industry what is expected of foods produced under properly managed operations. At the same instance, flexibility is given to industry to use different production, manufacturing, distribution, marketing, and preparation approaches for meeting the risk-based metrics in accord to their technical capabilities and preferences (CAC 2007a).

Where setting a FSO is considered a feasible risk management option, the purpose of an FSO set by a competent authority of a country may be to

- relate the expected level of operational control over a particular hazard associated to a certain food to a policy level of currently achieved public health protection that is relevant for local and or international trade, i.e. an ALOP/TLR.
- drive necessary improvement in the food safety status of a pathogen-product combination on the basis of a forward looking public health goal or the need to mitigate a food safety status that is deemed unacceptable, for instance targeting more stringent food safety control(s) by the industry or change in behavior of consumers

In both cases, industry is expected to put in place operational food safety control systems that deliver a level of food safety in line with the FSO, by establishing one or more appropriate POs, PCs and other control measures on the basis of coordinated interaction various food business operators in the food supply chain of concern.

POs may be set by government to guide a particular industry in establishing appropriate control measures at specific points in the food supply chain for instance in such cases where the government considers that this industry typically may not have the means to establish such measures themselves or where these measures are of critical importance to the performance of the overall food supply chain.

Competent authorities may include reference to FSOs/POs in their food standards or guidelines, but since the FSO specifically relates to the time of consumption, it is unlikely that a competent authority would use FSOs as regulatory metrics at the operational level due to the difficulty in verify that control at this point in the food supply chain is being met. Therefore, it is more likely that for operational purposes, competent authorities articulate POs where appropriate to communicate to the industry and other stakeholder what their risk-based food safety expectations are at specified points in food supply chains.

As agreed at the level of Codex Alimentarius (CAC 2007a), setting FSOs is the sole prerogative of competent authorities, who may base the values for this metric on explicit or even implicit public health targets, epidemiological data or insight in the hazard characterization for the hazard at hand. Likewise, POs can be derived from public health targets or using other relevant tools/information on the dynamics of a hazard between the point of consumption and the upstream point in the food supply chain where a PO is considered as a feasible risk mitigation option. Use of quantitative risk assessment approaches developed for the relevant pathogen in a particular food, preferably developed for/by a competent authority, has been advocated by several authors for linking FSOs and/or POs to ALOPs (e.g. Nauta and Havelaar 2008; Tenenhaus-Aziza et al. 2014; Walls 2006; Zwietering 2005).

Industry may find it useful to establish one or more POs along the food supply chain to coordinate overall management and ensure that, where a FSO has been set by government, the food safety status of a food expected at the point of consumption is duly achieved. Individual food business operators working in and along the food supply chain may choose to use any of the methods that competent authority use when deriving POs from FSOs. Food business operators can establish a PO on the basis of either an FSO set by a competent authority, or from an evaluation (usually quantitative) of the fate of the hazard in the specific food supply chain, ultimately resulting in an estimate of the risk (Zwietering et al. 2014).

Thus, the concepts of FSO and PO have very practical value and can be commonly understood and applied by industry and regulators, alike. Since FSO/PO do not specify how compliance is achieved, the concepts offers considerable flexibility to food business operator(s) involved in the particular food supply chain. This would enable one operator to use formulations, equipment and procedures that differ from other operators as long as the FSO/PO is met. Furthermore, there can be a high level of confidence in the acceptability of food being produced by operations that have been designed and validated to meet the relevant FSOs/POs. Foods from such operations need seldom be tested for the relevant pathogen(s) to verify compliance. Instead, verification can be achieved through record review and observation of GMP/GHP and HACCP (see Chap 4).

Since the adoption of the concepts of FSO and PO, many studies on their application for a diverse range of pathogen-product combinations have been published through peer review processes (e.g. Anderson et al. 2011; Crouch et al. 2009; De Cesare et al. 2014, 2015; Gkogka et al. 2013; Manfreda et al. 2014; Membre et al. 2007; Nauta and Havelaar 2008; Paulsen et al. 2009; Perni et al. 2009; Sosa et al. 2011; Skjerdal et al. 2014; Tenenhaus-Aziza et al. 2014; Tromp et al. 2010; Tuominen et al. 2007; Uyttendaele et al. 2006; Walls 2006) or are available in the public domain (e.g. Buchanan et al. 2006; DaPaola et al. 2006; Butler et al. 2006).

In summary, establishing risk-based metrics such as FSO and PO offers many advantages for both control authorities and industry because they can be used to:

- translate a public health goal to a measurable level of control upon which food processes can be designed so the resulting food will be acceptable
- validate food processing operations to ensure they will meet the expected level of control
- assess the acceptability of a food operation by control authorities or other auditors
- highlight food safety concerns, separate from quality and other concerns
- force change in a food commodity and improve its safety
- serve as the basis for establishing microbiological criteria for individual lots or consignments of food when its source or conditions of manufacture are uncertain.

It is not necessary to establish an FSO for all foods or all known hazard-food combinations. In some cases the potential microbiological hazards associated with a food represent so little risk at consumption that an FSO is not needed (e.g., granulated sugar, sweetened condensed milk, most breads, pineapple, carbonated beverages). In other cases the sources of a pathogen are so variable that identifying the foods for which FSOs should be set is not possible. An example of the latter is shigellosis which can be transmitted by many routes, most of which are more important than food (e.g., water, person-to-person), and it is unpredictable which specific food may next be implicated.

The FSO also becomes useful when the safety of new products is evaluated. When placing new products or novel foods on the market, their safety should be substantially equivalent to existing similar products.

Investigation of foodborne disease continues to identify new pathogens and new pathogen-food combinations. The emergence of listeriosis as a foodborne disease during the 1980s as a result of outbreaks traced to coleslaw and Mexican-style cheese is an example of a recently recognized foodborne pathogen. The finding that non-pasteurized juices and raw vegetables can be vehicles for *E. coli* O157:H7 is an example of a new pathogen-food combination. In such situations a quick decision may

be necessary to prevent more cases or outbreaks. Establishment of an interim FSO could be an initial step to communicate to the food industry, or exporting countries, the maximum level of a hazard at consumption that is considered to be acceptable. As further knowledge about the hazard, the food and conditions leading to illness become available and effective control measures can be determined, that interim FSO can be adjusted.

As noted before, FSOs/POs can be used to force change in an industry and enhance the safety of certain products. Many examples could be cited where epidemiologic data indicated certain foods were linked to foodborne illness. In response to this information governments used various mechanisms at their disposal to bring about the changes necessary to reduce or eliminate the risk of disease. In some cases, modifications in primary production or manufacturing practices may have been necessary, including the adoption of new or more reliable technologies, while in other situations risk could be effectively reduced for instance by consumers or food service operations changing behavior or practices. The establishment of an FSO/PO could be used by risk managers in government to communicate to impacted stakeholders the level of control expected and, thereby, forcing the required change.

The WTO/SPS agreement recognizes that governments have the right to reject imported foods when health of the population may be endangered. The criteria used to determine whether a food is considered to be safe or unsafe should, however, be clearly conveyed to the exporting country (transparency) and should be scientifically sound. Integral to the treaties is the concept of "reasonableness", a requirement that is inherent to the establishment of realistic FSOs. An exporting country can contest an FSO that does not reflect conditions existing in the importing country, and argue that the FSO is an unjustified trade barrier. However, because an FSO also reflects commercialization conditions, eating habits, preparation and use practices, FSOs may vary considerably between countries. Nevertheless, a country cannot demand that imported foods are "safer" than similar domestically produced foods. For example, if the tolerance for aflatoxin in domestically grown and processed peanuts is 15 μg/kg, then imported peanut products cannot be rejected if contaminated to the same or a lesser concentration. FSOs provide a means for implementing the concept of equivalence in Article 4.1 of the Agreement on Sanitary and phytosanitary (SPS) measures of WTO." Members (countries) shall accept the SPS measures of other Members as equivalent, ..., if the exporting Member objectively demonstrates ... that its measures achieve the importing Member's appropriate level of sanitary or phytosanitary protection."

2.6 Establishment of an FSO/PO Based on a Risk Evaluation by an Expert Panel

Some risk evaluations must be conducted prior to the establishment of an FSO/PO. Where the available time and resources limit the ability of risk managers to commission a detailed qualitative or (semi-) quantitative risk assessments, FSOs/POs might rather be based on the advice of an expert panel. For those hazard-food combinations where complexity is not a major issue, but where there is good agreement on the factors determining risk and options to mitigate risk, where necessary, there may not be a need to do such detailed risk assessments.

FSOs contain three elements; namely, the hazard, the food and the frequency and/or concentration of the hazard that is considered tolerable. Some basic knowledge is necessary before an FSO can be established. For this reason panel members should be selected based on their knowledge, experience and access to information that can ensure these basic needs are met. At a minimum the panel must have knowledge about the microbiological hazard (e.g. infectious agent, toxic metabolite), its potential source(s), relevant conditions along the food chain that lead to foodborne illness, and the range of host susceptibilities. The relationship between the microbiological hazard, the food and the disease may be elucidated through a combination of passive and active epidemiological programs, case-control studies, and other pertinent public health studies as described above. Investigations of foodborne

illness should also provide information about whether a certain population is at higher risk and the severity of the disease. This knowledge should be supplemented with data derived from laboratory research and from steps in the food chain that may be important relevant to the pathogen-food combination of concern. Records of foods processed for safety may provide useful data concerning the level of consumer protection normally achieved. This knowledge can form a solid basis for a risk evaluation and determination of an FSO and/or PO.

If it is known, for example, that the source of a pathogen (e.g., *S. aureus*) is humans and animals, and that growth on cooked ham during storage at room temperature to a high cell concentration (e.g., 10^6 cfu/g) is necessary for toxin production, then this information can be used in establishing an FSO/PO value. Additional information about the concentration of toxin (i.e. dose) required to cause illness would be needed before a meaningful FSO/PO could be developed. In this case it would be necessary to determine whether to base the FSO/PO on the concentration of *S. aureus* or on the concentration of staphylococcal enterotoxin in the food.

As another example, the cases of listeriosis in a number of countries have been between 0.1 and 1.3 per 100,000 per annum (Table 2.1). When the vehicles were identified, high numbers of the organism were present in the food (McLauchlin 1995, 1996; Anon. 1999). As *L. monocytogenes* is ubiquitous and low numbers are prevalent and ingested daily by consumers with no adverse effect, ICMSF, acting in the capacity of an expert panel, suggested that such low numbers are unlikely to pose a risk to healthy consumers and, therefore, proposed an FSO of 100 cfu/g at time of consumption for foods (ICMSF 1994).

2.7 Evaluation of Risk by Detailed Qualitative/Quantitative Risk Assessments

The purpose of a detailed qualitative or (semi-)quantitative risk assessment is the same as that of the risk evaluation by expert panels discussed above, namely to provide scientific advice to the risk managers who will use the information to decide upon the risk management option(s) that will be implemented to achieve the desired level of consumer protection.

Detailed qualitative or (semi-)quantitative risk assessments involve persons similar to those of the above mentioned expert panels, covering various relevant expertise areas, and importantly also experts with mathematical/statistical and/or computing skills in the case of (semi-)qualitative assessments. Such assessments typically come with longer timelines for establishing risk evaluations, for instance because of the inherent complexity of the food safety problem, the need to collect and review pertinent data and information, to do some level of investigation into key data gaps or establish and validate new data handling models.

2.7.1 Quantitative Risk Assessment

Quantitative assessments will normally be undertaken for complex situations, when there is substantial uncertainty about where control can best be exercised or the effectiveness of various control options, and/or when there is substantial disagreement among stakeholders concerning the level of control needed to achieve a tolerable level of consumer protection.

Microbiological risk assessment (MRA) comprises four basic steps: hazard identification, exposure assessment, hazard characterization and risk characterization (CAC 1999) and guidance on MRA and several of the steps is available from JEMRA consultations (FAO/WHO 2000, 2002, 2003, 2008a, 2009a). Each step involves a systematic process for collecting, assembling and providing the necessary knowledge to evaluate the public health significance of a microbial hazard in food. The final

outcome of the four steps is a risk estimate, i.e. a measure of the magnitude of risk to a population of consumers or the risk per serving to a consumer attributable to the food. The estimates are derived mathematically by calculating the likely frequencies and/or concentrations of the hazard in food at the time of consumption, combined with an estimate of the probability that disease will occur after the food is consumed. Ideally, risk estimates are given with the attendant uncertainties and a view on variability. Where appropriate for information of the risk manager, different scenarios of factors contributing to the consumer risk or of options to mitigate risks may be developed into risk estimates.

Of necessity, assumptions will be made during the assessment when data or other information is missing or incomplete. Data used and assumptions made should be clearly documented, and their effect on the final risk estimate clearly stated. It is also important that risk assessors identify, describe, and, if possible, quantify sources of variability and uncertainty that affect the validity of the risk estimate.

2.7.2 Hazard Identification

The first step of risk assessment, "hazard identification", assembles the knowledge about the pathogen and/or food in question, and its association with adverse health effects. Sometimes epidemiological data clearly identifies that foodborne transmission plays a role and which foods are implicated. Conversely, if a particular food is suspected, epidemiological and microbiological data may indicate which pathogens have been, or potentially could be, associated with the product. Epidemiologic data from disease monitoring programs, or investigations of foodborne outbreaks are often the first indication of a food safety problem with adverse effects associated with the pathogen being relatively well documented. Information may also come from animal disease monitoring when the pathogen is a zoonosis.

2.7.3 Exposure Assessment

Exposure assessment estimates the prevalence and levels of microbial contamination of the food product at the time of consumption and the amount of the product consumed at each meal by different categories of consumers. Programs for nutrition and consumption habits are often available nationally to gauge food intake and can be used to estimate exposure. The exposure assessment may be limited to measurements of pathogen levels at the time of consumption. However, models are developed that estimate how factors such as prevalence of pathogens in raw ingredients, the potential growth of the pathogen in the food, and impact of handling and preparation practices, affect the frequency and levels of pathogens consumed (PPP or Product/Pathogen/Pathway ("Farm to Fork") analysis). Data from base-line surveys of pathogens in foods and predictive microbial modeling techniques have proven to be valuable sources for deriving probable exposure estimates for pathogenic bacteria (ICMSF 1998b). Substantial amounts of information on microbial levels have been accumulated in food inspection data in many countries and could provide an additional source of information on the microbiological status of foods just before consumption.

The sensitivity, specificity and validity of sampling and testing methods used to collect empirical information should be considered to assure that results from different studies are comparable. Some apparent differences in pathogen prevalence in the food chain may be attributable to under-reporting or methods employed; however, there may be real variation due to ecological situations, or differing food safety control measures and animal health control programs. For example, food distribution systems vary from country to country with respect to temperature control. Exposure assessments should also consider differences in the cultural, social, economic or demographic structures of societies, which may influence consumption patterns and practices.

2.7.4 Hazard Characterization

Hazard characterization describes the severity and duration of adverse health effects that may result from the ingestion of a microorganism or its toxin in food. A dose-response assessment provides an estimate of the probability that disease/illness will occur in a certain category of consumers after exposure to a certain number of a pathogenic microorganisms and/or their metabolites/toxin (i.e., dose). The consequences of being exposed to a microbial pathogen or microbial toxin in a food will vary, ranging from no discernible effect to infection (colonization and growth in the intestinal tract) without symptoms of illness, to acute illness (usually gastroenteritis, but sometimes septicemia and meningitis), to long-term effects or sequellae (chronic illness such as reactive arthritis, Guillain-Barré syndrome or hemolytic uremic syndrome), to death. The likelihood that exposure to a particular dose (i.e., number of cells) of a specific pathogen may have any one of these consequences is dependent on three factors:

- characteristics of the micro-organism itself, (e.g., mechanism(s) of pathogenesis, virulence factors, ability to resist the host's defenses) that vary among strains and may be altered by prior conditions;
- the susceptibility of the host (e.g. immune status, predisposing conditions, age) and
- characteristics of the food in which the pathogen is carried (e.g., fat content, acidity, or other factors that affect the organism's capacity to resist acidity of the stomach, competing bacteria in the food etc.).

In practice, estimates of the numbers of pathogen that may cause illness and the severity of illness relative to dose are derived from experimental studies with humans, from animal models and epidemiological data (and accumulated knowledge and experience) (See Chap. 8).

The final risk characterization (see below) is dependent on being able to derive the relationship between the frequency of exposure of the population (or subpopulation) with various numbers of the pathogen in the food at the moment of consumption and the number of illnesses (e.g., gastroenteritis, death) per annum has to be established. Figure 2.3 depicts different possible dose-response relationships for *L. monocytogenes* that were deduced as part of a JEMRA study (FAO/WHO 2003). It is apparent from this and other studies that variability and uncertainty may be factors that need to be dealt with in hazard characterization.

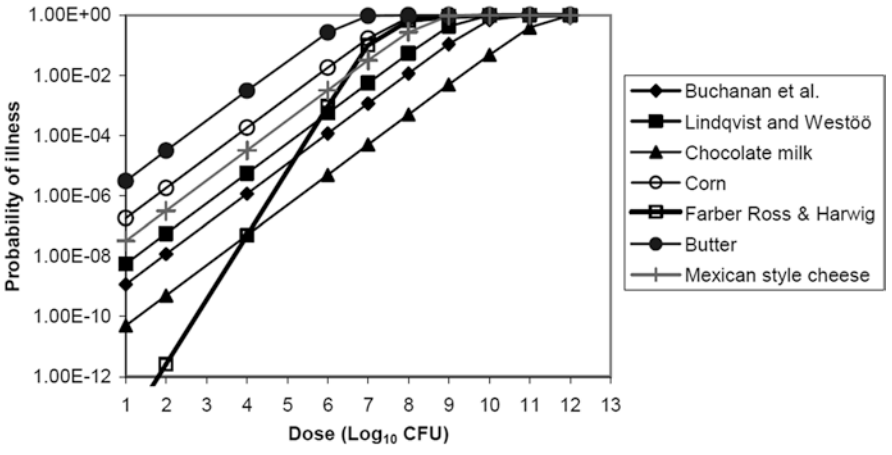

Fig. 2.3 A comparison of 8 dose-response curves for morbidity estimated for *Listeria monocytogenes* exposure through different food products and for a number of subpopulations by the JEMRA panel on risk assessment of *L. monocytogenes* in ready-to-eat foods. For full details see (FAO/WHO 2004)

2.7.5 *Risk Characterization*

Risk characterization combines the information generated in hazard identification, exposure assessment and hazard characterization to produce a complete picture of risk. The result is a risk estimate that, for instance, is an indication of the level of disease in a population per unit of time (e.g. number of cases per 100,000 persons per year) resulting from the given exposure. An example of a risk characterization is presented in Fig. 2.4, which is from a study conducted in New Zealand on the public health risk associated with the consumption of untreated raw milk, considering a number of risk scenarios related to different milk production and handling practices such as the temperature of milk storage at the farm level (MPI 2013).

Whenever possible, the resulting risk estimate (or risk estimates when different scenarios or subpopulations are considered) should be compared with epidemiological data, or other reference information, to assess the validity of the risk assessment's models, data, and assumptions. The risk estimate(s) should reflect a distribution of risk that represents the range of contamination of a food product, factors that might affect growth or inactivation of the pathogen, and the variability of the human response to the microbial pathogen as well as the uncertainty in the estimate(s).

Risk characterizations should also provide insights about the nature of the risk which are not captured by a simple qualitative or semi-quantitative statement of risk, e.g. identifying the most important factors contributing to the average risk, the uncertainty and variability of the risk estimate, and gaps in data and knowledge. The consequences of any default assumptions provided to the risk assessment team should be documented. The risk assessor may also compare the effectiveness of alternative methods of risk reduction, enabling the risk manager to consider risk management options.

Where appropriate, the resulting risk estimate(s) may be compared to the tolerable level of risk decided upon by governmental risk managers and if a risk estimate is higher than that which can be tolerated, obviously, actions should be taken to reduce the risk.

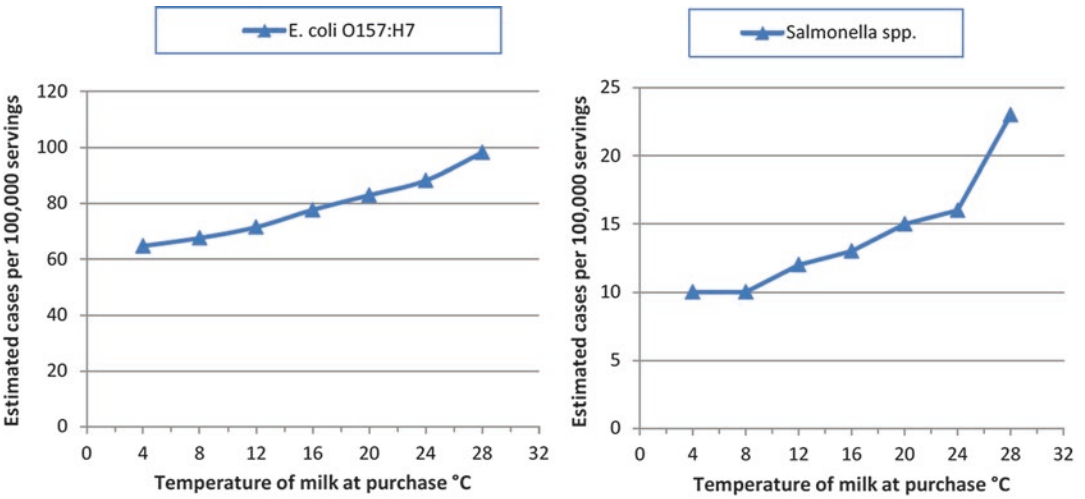

Fig. 2.4 Risk characterization curves based on estimates of the risk of illness in the population resulting from *E. coli* O157 and *Salmonella* spp. in raw milk, depending on the temperature of milk purchased from the farm vat (MPI 2013)

2.7.6 *Mathematical Approaches*

Quantitative risk assessments use mathematical models to estimate risk as a function of one or more inputs. Point-estimates, or single values such as the means or maximum values of variable data sets, have been used historically to generate a single numerical value for the risk estimate.

Until recently, the most common approach was to use either the means or worst-case (95 percentiles) estimate calculated from the available data for each step of the assessment. These values were then used to calculate an over-all mean or "worst-case" single value estimate (e.g., 1 per 100,000 exposures will result in illness; 100 cases/100,000 population). Such risk estimates were termed "deterministic" or "point estimate" risk assessments. A major shortcoming of these approaches is that the variability of diverse and dynamic biological phenomena is ignored and consideration is not allowed for how much uncertainty there may be about the data and how it may influence the risk estimate. Deterministic risk assessment may be coupled with the use "uncertainty factors" similar to the "safety assessment" paradigm used for managing the risks associated with chemical contaminants.

Probabilistic assessments represent all the information available for each parameter (i.e., information or dataset about a factor that is important in determining risk) described as a distribution of possible values. A mathematical description of the production and consumption of a food using probability distributions is very difficult to calculate analytically. While some analysis is practical on very small and simple models, a compound model of food production involving pathogen growth, destruction and infection is too complex to interpret without computational tools. Probabilistic risk assessments for food safety are feasible using commercial software. Monte Carlo simulation is a computational tool that aids in the analysis of models involving probability distributions.

Figure 2.5 shows an example of a probabilistic risk characterization outcome in the form of a ranking of population level risks associated to different products within a product category. Note that in this example an indication is provided of the range of variability and uncertainty of the estimates by the upper and lower bounds (5th and 95th percentile values).

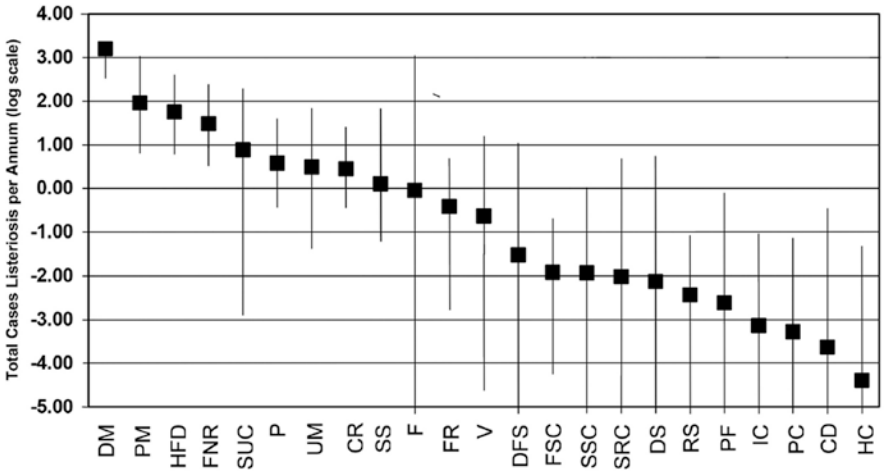

Fig. 2.5 Risk characterization in the form of a risk ranking of predicted cases of listeriosis per annum associated with a range of food categories for the total United States population; the black box indicates the median predicted number of cases of listeriosis and the bar indicates the lower and upper bounds (i.e., the 5th and 95th percentiles). Full details in FDA/FSIS (2003). *DM* Deli meats, *FNR* Frankfurters (not reheated), *P* Pâté and Meat Spreads, *UM* Unpasteurized Fluid Milk, *SS* Smoked Seafood, *CR* Cooked Ready-To-Eat Crustaceans, *HFD* High Fat and Other Dairy Products, *SUC* Soft Unripened Cheese, *PM* Pasteurized Fluid Milk, *FSC* Fresh Soft Cheese, *FR* Frankfurters (reheated), *PF* Preserved Fish, *RS* Raw Seafood, *F* Fruits, *DFS* Dry/Semi-dry Fermented Sausages, *SSC* Semi-soft Cheese, *SRC* Soft Ripened Cheese, *V* Vegetables, *DS* Deli-type Salads, *IC* Ice Cream and Frozen Dairy Products, *PC* Processed Cheese, *CD* Cultured Milk Products, *HC* Hard Cheese

2.8 Establishment of an FSO Based on Quantitative Risk Assessment

Risk assessments can help to identify how the frequency and/or concentration of a microbiological hazard in a food or group of foods can influence the incidence of a disease. There is a relationship between the level of a hazard in a food (the dose) and the incidence of the disease it causes in a given population (the response). Likewise, the illness response of individual consumers likely relates to the dose of the hazard ingested through a food item. However, the relationship(s) generally is/are rather uncertain (since meaningful controlled consumer exposure studies are ethically unacceptable) and variable (because of inherent biological variability of the hazard as well as individual consumers). Consequently, capturing the dose response relationship in a risk assessment study is rarely straightforward. Nevertheless, informed choices will have to be made to represent the impact of a hazard on consumers by a hazard characterization curve (e.g., Fig. 2.3) such that risks can be determined. The slope of a hazard characterization curve is specific to the hazard, the food, the illness and the (category of) consumers for which the curve has been determined. If such curves are available for the incidence of disease for a specific pathogen-food combination, an ALOP or TLR value can be positioned on the y-axis and the corresponding level of the hazard, which represents then the FSO value, can be obtained on the x-axis. However, the uncertainties and variability that underlie establishing hazard dose – consumer response relationships may oblige risk managers to take somewhat cautionary approach and select an FSO value that is more stringent, i.e. set at a lower level of the hazard.

In many situations the public health goal is to approach "zero cases" for a given pathogen-food combination (e.g., no cases of botulism from commercially canned shelf stable low acid foods). Although this is not always feasible, it may drive adoption of more conservative FSO values. However, the costs to realize this goal may be higher than a society will tolerate (see Sect. 2.8). It must be remembered that the FSO will reflect the balance between the costs associated with reducing risk and the costs associated with accepting risk.

When interpreting hazard characterization curves such as those depicted in Fig. 2.3, it is important to note that for infectious agents the relationship between consumer risk and the hazard level may reach zero seldom, if ever, unless the hazard has been eradicated. Instead, as the predicted incidence falls below unity, the population size and the timeframe need to be adjusted. Even when the risk to a population falls to the level where the predicted value is less than 1 case per year does not indicate that there is zero risk. For example, a predicted incidence of 0.2 cases per year would be more appropriately expressed as 2 cases per 10 years.

Ideally, an FSO would be based on the frequency and/or concentration of a pathogen in a food that would not produce disease. This would be equivalent to finding a no-effect dose, the value that is used for setting tolerable levels of daily exposure for acutely toxic chemicals. While certain foodborne pathogens may have definable threshold levels below which they pose no appreciable risk to the (generally healthy) consumer, most risk assessment dose-response models for infectious pathogens are based on the assumption that there is a chance, however remote, that one single cell may cause disease. On the contrary, for particular toxigenic foodborne pathogens (i.e., microorganisms that cause disease through the production of a toxin) it is assumed that there is a threshold concentration of cells below which the microorganism does not produce sufficient toxin to cause an adverse effect. As an example, low levels (i.e., $<10^4$ cfu/g) of *S. aureus* in a food generally do not represent a direct risk to humans, whereas higher levels ($>10^5$ cfu/g) do so through the production of enterotoxin to levels that will probably cause staphylo-enterotoxicosis. Evidently, establishing FSOs is substantially more complex when a no-effect level cannot be assumed, however, the basic process for establishing a FSO remains the same.

2.9 Stringency of FSOs/POs in Relation to Risk and Other Factors

Examples of hypothetical FSOs and POs are provided throughout this book to describe the FSO/PO concepts and how they can be used as food safety management tools based on assumed quantitative relationships between tolerable hazard frequencies/concentrations that reflect risk.

Some of the suggested FSO/PO values may be more restrictive for a certain food-pathogen combination, even though another food-pathogen combination may be of greater risk.

The commission provided values for FSOs in this book only as "illustrative examples" for discussion purposes, since only competent authorities can establish or endorse FSOs. Several different pathogen-food combinations are considered in Chaps. 14, 15, 16, 17, 18 and 19, and the FSO values suggested are aflatoxin in non-ready to eat peanuts (FSO < 15 μg/kg), *Salmonella* and *Cronobacter* in powdered infant formula (FSO < 1 cfu/kg), *L. monocytogenes* in ready to eat deli meats that do not support growth (FSO ≤ 100 cfu/g), *E. coli* O157:H7 in leafy green produce (FSO ≤ 1 cfu/66 kg), norovirus and hepatitis A viruses in oysters (FSO ≤ genome copy/60 g) and *C. jejuni* in cooked poultry meat (FSO ≤ 1 cfu/28 kg).

Relative risk is not reflected in the stringency of the FSOs suggested. For example, recognizing that as many as 15% of the USA population consumes undercooked ground beef, the FSO for ground beef is much less stringent than the FSO for powdered infant formula. In the case of powdered infant formula, there has been about 30 years of commercial experience in identifying and implementing the necessary controls for *Salmonella*. The FSO is commercially achievable with current technology and equipment and processes can be validated as meeting an FSO of this stringency. The available control measures are much less effective for *E. coli* O157:H7 from slaughter through grinding (mincing). In the absence of an effective kill step (e.g., irradiation, high pressure) a more stringent FSO is not achievable.

Ideally, as risk increases the corresponding FSO/PO would become more restrictive but still proportional comparing risks across different pathogen-food combinations. Due to the many factors influencing the decisions of risk managers it is uncertain whether an internationally recognized system can be developed for FSOs that would be based on relative risk ranking.

2.10 Summary

FSOs and/or POs for hazard-food combinations provide new metrics for governmental risk managers that can help make more explicit to industry and other stakeholders what is expected in terms of the food safety status of particular foods and food categories at the point of consumption or at earlier points in a food supply chain. Industry may find it beneficial to use the PO concept in arranging their food safety management system(s) based on GMP/GHP and HACCP such that an FSO set by government is met. Before the concepts of these risk based metrics were adopted, rarely has there been any quantitative, outcome-based expression of the expected level of safety of a food, i.e., valid at the time that the consumer eats it and when hazards associated to such a food may actually cause consumer exposure possibly resulting in illness. Typically, reliance has been placed most commonly on using process or product criteria valid at a particular point in a food supply chain, for instance specified in regulations or industry guidelines, or in end product testing in compliance with microbiological criteria. While microbiological criteria will continue to be used, the concepts of FSO/PO may guide designing food safety management systems to achieve specific food safety goals based on an expected level of consumer protection. This approach will further strengthen the scientific basis of existing food safety management systems, and the suite of control measures underlying such systems.

Government risk managers considering the establishment of an FSO (and/or PO) for the enhancement of the safety of a certain food should verify that the metric is achievable. This should include a process communication with stakeholders that must necessarily be iterative. Information must be exchanged between those proposing an FSO/PO (and possibly particular control measures) and the affected industry, consumers, etc. If the FSO is not achievable and/or the possible control measures are not possible, for example, with existing equipment, then some adjustments may be necessary. The adjustments may involve modifying the FSO/PO and/or changing equipment or industry practices.

The process of verifying that an FSO/PO is achievable, and then establishing and validating the effectiveness of control measures, is discussed in the next chapter.

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Chapter 3
Meeting FSO and PO Through Control Measures

3.1 Introduction

FBOs have to go through two important steps before bringing a new food product to market. They first develop a validated product and process design for their food product that will meet the relevant objective for safety. Then, this design is implemented operationally, at the point in the food supply chain that the FBO is operating at and/or is responsible for, using a food safety management system that is based on good practices (also known as *prerequisite programs*) and the principles of HACCP.

Jointly, prerequisite programs and HACCP manage the control measures that ensure control of product safety at the relevant step in the food supply chain. As part of the implementation step, the proper functioning of the food safety system is validated before food is brought to market. During day-to-day operation of the food operation, the proper functioning of the food safety system is verified. Both validation and verification relate to particular control measures that are of key importance to assure control of safety for products going to market.

Effective application of GHP and other prerequisite programs, as appropriate, provides the foundation upon which HACCP systems are developed and implemented. Failure to maintain and implement GHP or other prerequisite programs can negate a HACCP system and result in production of unsafe food.

As discussed in Chap. 2, the modern approach to ensuring food safety is that effective food safety management systems of the Industry are designed to meet Food Safety Objectives (FSOs) and/or Performance Objectives (POs) established by competent (control) authorities through appropriate risk management processes. Should such objectives not have been established by authorities, food business operators (FBOs) may set their own objectives, either as POs or other suitable formats. In any case, objectives are achieved in real operations through the application of one or more control measures intended to prevent, eliminate or reduce microbiological hazards, such as those discussed in this Chapter.

Note the definitions of FSO and PO as agreed at the level of Codex Alimentarius (CAC 2015):

– Food Safety Objective (FSO): “The maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP)”,
– Performance Objective (PO): The maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption that provides or contributes to an FSO or ALOP, as applicable”.

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3.2 Control Measures

While microbiological criteria have played an important role in defining what microbiological specifications of food have been considered acceptable, in isolation they cannot be considered genuinely effective for the control of microbial hazards. In ICMSF Book 4 (ICMSF 1988) it was recognized that more emphasis should be placed on the use of selected, targeted control measures and less on microbiological testing of food. ICMSF Book 8 (ICMSF 2011) further stressed the role of testing to verify the effectiveness of a food safety management system, and the use of data for assessing process control and product acceptance, providing practical examples of useful approaches applied to a range of product categories and for a variety of foods and processing environments. For each product category, the most effective control measures are being suggested that are based on the principles described in ICMSF (2002), of which this book is a revised version. Microbiological testing alone is not being recommended by ICMSF as an effective control measure and food manufacturers should rather control their control measures, processes and the conditions of their operations to assure that their products would meet the relevant microbiological criteria if sampled.

In the present day, many food manufacturers and other food business operators around the world have indeed adopted food safety assurance approaches to ensure the safety of finished products. These approaches rely on the selection of sound product designs and processes for producing the foods and on control of food operations by use of prerequisite and HACCP programs. In doing so, explicit safety expectations, e.g. FSOs/POs or microbiological criteria, are being met.

Historically, major advances in consumer protection have resulted from the development and implementation of selected, targeted *control measures* at one or more steps along the food chain, from the farm up to the consumer. These advances have followed periods of extensive investigation to gain the information necessary to understand the pathogens (e.g. sources, life cycle, parameters influencing growth, survival, death, or metabolite production).

Control measures are the actions and activities used to prevent, eliminate or reduce a food safety hazard to a tolerable level. They generally fall into three categories:

Controlling initial levels

- Avoiding foods with a history of contamination or toxicity (e.g., raw milk, raw molluscan shellfish harvested under certain conditions).
- Selecting ingredients (e.g., pasteurized liquid eggs or milk).
- Using microbiological testing and criteria to reject unacceptable ingredients or products.

Preventing increase of levels

- Preventing contamination (e.g., adopting Good Agricultural Practices during primary production or implementing Good Hygiene Practices that minimize contamination during processing operations, such as slaughter, separating raw from cooked ready-to-eat foods, implementing employee practices that minimize contamination, using aseptic filling techniques).
- Preventing growth of pathogens (e.g., chilling and holding temperatures, pH, a_w, preservatives).

Reducing levels

- Destroying pathogens (e.g., freezing to kill certain parasites, sanitizers, pasteurization, irradiation).
- Removing pathogens (e.g., washing, ultra-filtration, centrifugation).

One or more of the above activities may be necessary to control a hazard at a particular step in a food supply chain. In addition, one or more control measures may be applied at different steps along the food supply chain, in order to eliminate, prevent or reduce a hazard to an acceptable level. Each participant along the food chain has a responsibility to apply those control measures that contribute to

providing safe foods. Even at the final food preparation and consumption stage, measures can be taken to avoid recontamination or growth of micro-organisms. For instance, good hygiene is an important control measure at the stage of final food preparation in the home or in a food service operation as it is essential to control cross contamination. As such, the education of food handlers in the home or in food service in the necessary skills and knowledge about good hygiene in itself can be seen as an important control measure.

Regarding FBOs, the various control measures they apply generally fall into two sets of programs:

– good practices such as Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP) and/or Good Hygiene Practices (GHP) and
– programs based on the principles of Hazard Analysis Critical Control Point (HACCP).

The good practices mentioned in the first program are part of a broader suite of systems collectively referred to as pre-requisite programs (Chap. 1). Good Hygiene Practices (GHP) can be viewed as the basic sanitary conditions and practices that must be maintained to produce safe foods. It also includes certain support activities such as raw material selection, product labeling and coding or recall procedures. Effective application of GHP provides the foundation upon which the second program, HACCP, is developed and implemented. The development of an effective HACCP system involves a systematic approach to the identification, evaluation, and control of all types of food safety hazards in a food operation.

It is important to note that HACCP is not implemented in lieu of prerequisite programs such as GHP. On the other hand, and failures to maintain and implement prerequisite programs can invalidate a HACCP system and result in production of unsafe food. It is necessary to consider the hazards that are most likely to occur in each particular food operation and pay particular attention to those elements of the prerequisite programs, in particular GAP and GHP, and as well as HACCP that will contribute most in controlling the hazards.

3.2.1 Good Hygiene Practices (GHP)

The General Principles of Food Hygiene (CAC 1997a) focus on the hygiene related prerequisite programs and describe the major components of GHP as:

- Design and facilities (location, premises and rooms, equipment facilities)
- Control of operation (control of food hazards, key aspects of food hygiene control, incoming material requirements, packaging, water, management and supervision, documentation and records)
- Maintenance and cleaning (maintenance and cleaning, cleaning programs, pest control systems, waste management, monitoring effectiveness)
- Personal hygiene (health status, illness and injuries, personal cleanliness and behavior, visitors)
- Transportation (general requirements, use and maintenance)
- Product information and consumer awareness (lot identification, product information, labeling, consumer education, handling/storage instructions)
- Training (awareness and responsibilities, training programs, instruction and supervision, refresher training)

As stated previously, effective application of GHP and other prerequisite programs provide for the foundation upon which HACCP systems are developed and implemented. Failure to maintain and implement GHP or other prerequisite systems programs can invalidate a HACCP system and result in production of unsafe food.

Effective control of a hazard in a food necessitates consideration of the components that generally (i.e. the prerequisite programs) and/or specifically (i.e. HACCP) are likely to have significant impact in controlling the hazard. For example, the risk of certain chemical hazards (e.g., paralytic shellfish poisoning, ciguatera toxin, scombroid poisoning) and biological hazards (e.g., pathogenic *Vibrio* spp.) being used in a range of finished goods all produced without a heating step to reduce risks, may be controlled through general incoming material requirements). Incoming material requirements related to biological hazards may be of lesser importance for a food product that will be cooked sufficiently to eliminate enteric pathogens that may be present, provided that sufficient cooking as a step at food preparation can be relied on. Thus, the various components of a pre-requisite program such as GHP do not carry equal weight in all food operations. It is necessary to consider the generic hazards that are most likely to occur across the foods produced in a particular operation and then apply those GHPs that will be most effective for controlling these hazards. This does not mean that the other components of GHP are ignored. Some, for example, may be very important to ensure a food meets established quality requirements.

In certain situations, selected components of GHP may carry particular significance and should be incorporated into the HACCP plan. For example, equipment maintenance and calibration are important for large continuous ovens used in cooking meat products. In this example, the procedure and frequency (e.g., monthly, quarterly) for conducting checks on heat distribution during cooking could be incorporated into the HACCP plan as a verification procedure. In addition, it is normally necessary to verify the accuracy of the thermometers used for monitoring oven temperatures during cooking.

There are several examples of outbreaks related to the failure to apply effective GHP. Table 12.1 in Chap. 12 details a wide range of outbreaks related to environmental contamination caused by poor design of equipment, poor separation of raw and cooked product, poor maintenance or cleaning of equipment or poor management of controls during transportation. In addition to environmental contamination, a large number of outbreaks from food service establishments can be attributed to poor personal hygiene (NSW Food Authority 2014).

Information on hygienic design of facilities and equipment, cleaning and disinfection, health and hygiene of personnel, and education and training are discussed in ICMSF book 4 (ICMSF 1988).

3.2.2 Hazard Analysis and Critical Control Point (HACCP)

The Codex document on the Hazard Analysis and Critical Control Points (HACCP) System and Guidelines for its Application (CAC 1997b) lists seven principles:

1. Conduct a hazard analysis
2. Determine the critical control points
3. Establish critical limits
4. Establish monitoring procedures
5. Establish corrective actions
6. Establish verification procedures
7. Establish record keeping and documentation procedures

The development of an effective HACCP system involves a systematic approach to the identification, evaluation, and control of food safety hazards in a food operation. HACCP plans specify the actions to be taken in a food operation to control food safety hazards. HACCP plans also specify records to be generated during the operation for use in verification that critical limits have been met at Critical Control Points (CCPs) in the operation. In the event a deviation occurs at a critical control point (CCP), the deviation should be detected in time to ensure that corrective actions will prevent

unsafe food from reaching consumers. This may necessitate collecting and analyzing samples from across the questionable quantity of food. The principles described in this text for sampling food can be applied to help assess the safety of a suspect lot and lead to appropriate disposition of the food (see Chap. 11, Sect. 11.2).

Codex Alimentarius has defined a critical control point as "a step at which control can be applied and that is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level" (CAC 1997b). The interpretation of what is considered an acceptable level has been left to the judgment of the HACCP team and respective competent authorities.

As discussed in the previous two Chapters, the concepts of FSO and PO can be used by competent authorities to communicate to food business operators the level of control necessary for a hazard to be reduced to "an acceptable level". Food business operators can also use the PO concept for coordination across a food supply chain between FBOs.

The production of safe food or food ingredients requires food business operators to selectively apply prerequisite programs such as GHP and the principles of HACCP to develop and implement a total food safety management system that will control the significant hazards in the food/ingredients being produced, handled or prepared.

Some risk management principles are best addressed through GHP measures (e.g., controlling the initial levels of a hazard through good hygiene), or control measures within other prerequisite programs, and others are clearly part of a defined CCP within HACCP (e.g., reducing the level of a significant hazard through a decontamination step). As mentioned above, in certain situations selected components of GHP may carry particular significance and should be incorporated into the HACCP plan. This is particularly relevant where the safe production of food requires a number of risk management control measures to be applied in combination to ensure a safe product.

For example, in the manufacture of fresh-cut, leafy green salads it is not possible to apply a single CCP such as a thermal pasteurization step to ensure safety without compromising quality of the product. Instead, to ensure a safe product requires a number of different risk management control measures, such as good agricultural practice, an effective washing step and control of the product distribution and retail conditions.

Over the last two decades, ICMSF and Codex Alimentarius have developed a risk-based framework for managing risks associated with foods, resulting in publication of new principles and guidelines for microbiological risk management (CAC 2007a). The use of these risk management principles provides a hierarchy of risk management metrics to guide the application of control measures throughout the food chain (see also Chap. 1).

Where regulatory authorities have stipulated their food safety expectations in terms of FSO or PO values for a type of food product, they will be concerned with whether a typical food safety management system implemented at a relevant point in the food supply chain for that group of products can meet the FSO/PO. To that end, they may provide default or 'safe haven' guidance to the industry on control measures that would be able to ensure that the stipulated objective(s) is met consistently, when adequately implemented by Industry. As noted before, such measures may be single control measures or combinations of control measures. Guidance may also be provided on potential issues related to processes and handling that Industry needs to be aware of in establishing the required effective food safety management system.

Food manufacturers design processes at a particular step in a food supply chain to meet the PO established by a government or the food business operator, ensuring that they can deliver the Performance Criterion (PC) across the step that is required to covert the hazard level coming into the step to the PO (CAC 2007a). The PC is "the effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a PO or an FSO" (CAC 2015). To achieve the PC, an operator selects one or more suitable control measures that have been duly validated (CAC 2008a).

The implementation of control measures will continue to be met through the use of pre-requisite programs and HACCP. However, it is expected that the use of the new risk management concepts will facilitate an understanding of different responsibilities for managing food safety through the food supply chain as well as better articulate in quantitative terms the stringency required of a given HACCP plan to achieve a defined outcome. It will facilitate the quantification of CCPs and critical limits within a HACCP plan with regards to the level or probability of a given hazard being properly controlled, which is a critical aspect of validation. As mentioned earlier, many outbreaks are related to failures in GHP and there are entire classes of foods for which current processing capabilities are limited to relatively small reductions (e.g. fresh produce). This has led to consideration of the use of the risk management framework in defining the performance of not only HACCP but also of GHP in managing risks (Buchanan and Williams 2013).

3.3 Confirmation that the FSO/PO Is Technically Achievable

Governments that are considering establishing an FSO and/or PO(s) for a particular food or food category should base their decision on a sound understanding of the suitability of such metrics to address a particular food safety problem or to improve a food safety situation as required, as well as the feasibility of implementing this. Important aspects of the decision therefore will be good insights in the preliminary process and product requirements and exchanges of information with relevant stakeholders, such as the affected industry.

Through stakeholder interactions, government risk managers should confirm that food business operators in the affected Industry (or other stakeholders) can technically achieve the FSO/PO established by government through an appropriate choice of one or more control measures that are run operationally through GHP and HACCP. Using the FSO/PO concept, government in principle does not specify how these metrics are met, leaving options open to Industry to choose the control measures best fitting their scope of operation, skills and business circumstances. Notably, the affected food business operators may be constrained by the choice of control measures that are within their scope, unless significant investments are made to expand.

If the proposed FSO/PO is agreed with the affected food business operators and/or other particular stakeholders to be achievable and is appropriately formalized by the competent authority, the affected parties will start to develop the final requirements for product and process to be implemented operationally to ensure that the objective(s) is/are met. Individual food business operators for instance will select the necessary control measure(s) for their food safety management system that is (are) validated to fulfill the overall Performance Criterion (PC) required to meet the PO/FSO for the step that the FBO controls, considering the incoming hazard level at that step (Chap. 2 and examples later in Chap. 3).

However, if the proposed FSO/PO is found not to be technically achievable with current skills and resources, a number of options could be pursued:

- Government may modify the FSO/PO such that it is possible for stakeholders (i.e. particular food business operators) to achieve these, but possibly not achieving the full level of improvement envisaged by the authority;
- Industry may be asked to invest in developing their skills and resources to the extent that it would be possible to meet the envisaged improvement level.

Modifications should result from discussions between government and other stakeholders, such as industry, aimed at achieving an optimal public health protection based on a tolerable level of risk. Where required and feasible, necessary improvements may be implemented over an agreed period of

time, in a phased manner. If no technically achievable solutions or acceptable modifications can be found, then it may be necessary to ban the product and/or the process.

As new information regarding a particular hazard and/or certain food products/categories emerges, FSOs/POs may be modified. As described in previous chapters, the FSO is the maximum level of a hazard that an authority tolerates in a food at the time of consumption and that in principle, according to Codex, provides or contributes to ALOP. By setting an FSO, competent authorities articulate a risk-based limit that should be achieved operationally within the food chain, while providing flexibility for different production, manufacturing, distribution, marketing, and preparation approaches. Because the FSO uniquely relates to the point of consumption, however, it is rather unlikely that a competent authority would set an FSO as a specific regulatory metric. After all, verification of the FSO being met in the frame of regulatory enforcement would be very unpractical at this point in the food supply chain.

The establishment of POs by a risk manager therefore provides an operational risk-based limit for a maximum hazard level allowed in a food at a specific point in the food chain, which should not be exceeded if one is to have confidence that the FSO or ALOP will be maintained. Since a PO is conceptually linked to the FSO and ALOP, the impact of the steps in the food chain both before and subsequent to the PO should be considered in setting its value. Figure 3.1 illustrates the use of PO in relation to potential control measures to achieve an FSO within a model food value chain.

To verify whether batches of food, food ingredients or other materials meet the FSO or PO at the relevant point in the food chain, microbiological criteria (MCs) can be used (CAC 2007a, 2013). Examples of how MCs may be derived from an FSO/PO can be found in Zwietering et al. (2015).

Similarly, the MC approach can be followed to verify objectives that are not necessarily identified as FSOs or POs. As one example, New Zealand has introduced a regulatory objective called the "Campylobacter Performance Target" to improve the food safety control systems for slaughter and dressing of broiler chickens with the aim of reducing *Campylobacter* levels on carcasses at the end of primary processing (Lee et al. 2014). An individual processor may decide on the GHP-based and hazard-based control measures that they apply to meet the performance target. For compliance verification purposes, the New Zealand government established a microbiological criterion for *Campylobacter* and test results are analyzed using a "moving window approach".

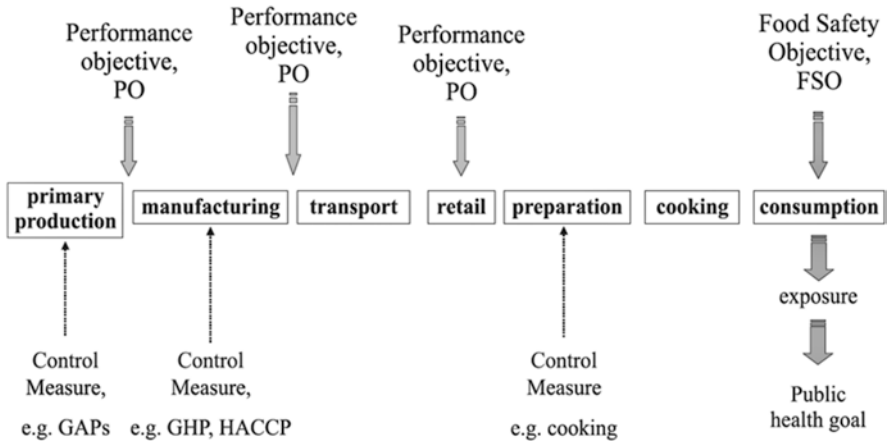


Fig. 3.1 Model food chain indicating the position of a Food Safety Objective and various other metrics derived from it, such as Performance Objectives and Performance Criterion (ICMSF 2006)

3.4 Importance of Control Measures

Historically, the major advances in consumer protection have resulted from the development and implementation of selected, targeted control measures at one or more steps along the food chain. Table 3.1 summarizes a number of foodborne diseases and measures that have been found to be most effective for their control. Certain biological hazards are influenced by environmental conditions that are beyond control of a food operator (e.g., ciguatera toxin in fish aquaculture or mycotoxin formation in crops in the field). For these hazards it has been essential to understand the effect of climatic conditions in the affected regions of the world. This information is used to determine when to screen for the hazard during periods of higher risk. Thus, to avoid these hazards, raw material selection is an important control measure. In addition, dehydration of certain crops can be used following harvest to prevent mycotoxin development during subsequent storage and distribution. For certain pathogens associated with livestock, selected on-farm practices have led to reductions in human disease. For example, brucellosis and trichinosis in the U. S. A. and Europe have been reduced by specific control measures (Figs. 3.2 and 3.3).

3.4.1 Examples of the Effectiveness of Control Measures

Foods have been preserved by salting, pickling, curing, heating, drying, etc., to save the food for those periods when they would not normally be available. These basic technologies have led to considerable diversity in the world's food supply. They also can be viewed as control measures that have evolved through experience and, when controlled correctly, have been found to yield safe food for later use. Table 3.2 summarizes the use of control measures that have been used to produce a wide variety of commercially prepared shelf-stable foods (Tompkin and Kueper 1982). In some cases, a single important control measure has been adequate; in most, however, combinations of factors have been used to achieve shelf stability.

Another means to demonstrate the importance of control measures is to examine trends of foodborne disease. For example, Fig. 3.2a shows the number of cases of human brucellosis per 100,000 population in the USA from 1939 through 1998 (CDC 2000a). A marked increase occurred during World War II that was attributed to greater movement of livestock among farms in response to the increased demand for meat, higher livestock prices and diversion of veterinarians to the war effort (Simms 1950). Similar disruptions in on-farm pathogen control practices would have been commonplace elsewhere in the world during that time period. During the period 1940–1947, human brucellosis was strongly associated with animal contact. For example in the state of Iowa, the rate per 100,000 population during this period was 562 for veterinarians, 276 for packinghouse employees, 59 for male farm workers, and 2 for the "no contact" group (Jordan 1950). Following the war, efforts resumed for control of brucellosis through a combination of on-farm practices and culling of livestock. Increased use of pasteurization of milk for manufacture of dairy products provided an additional important control measure that led to reduced consumer exposure.

Prior to 1940, the incidence of human trichinosis in the USA also decreased due to specific on-farm control measures, processing requirements in commercial meat establishments, and education of consumers regarding the necessity to adequately cook pork. After investigations demonstrated the significance of household kitchen garbage containing raw pork as a source of infection for pigs, regulations were implemented that required cooking garbage before feeding to pigs (Leighty 1983). Figure 3.3a shows the reduction of trichinosis in the United States after World War II. Trichinosis is an interesting example illustrating the differences in control measures adopted by the United States and Europe to achieve similar levels of protection. While in the United States control measures have been focused on heat destruction, e.g. by cooking, in Europe microscopic and later immunologic methods have

Table 3.1 Control measures commonly applied to foodborne diseases

Primary source	Group	Agent	Common vehicles	Control measures		
				Controlling initial level	Reducing level	Preventing increase in level
Human	Bacteria	*V. cholerae*, *S. typhi*	Untreated water, foods contaminated by infected food handlers.	Treat waste water, provide potable water for drinking and food preparation. Educate food handlers in proper personal hygiene.	Cooking	Pathogen increase in the implicated food is not an important factor.
		Shigella	Foods contaminated with untreated waste water or by infected food handlers.	Educate food handlers in proper personal hygiene. Exclude infected persons from high risk positions (eg, food preparation, day care centers).	Cooking	Pathogen increase in the implicated food is not an important factor.
	Virus	Hepatitis A, SRSV, Norwalk virus	Ready-to-eat foods, bivalve molluscs.	Treat waste water, provide potable water for drinking and food preparation. Educate food handlers in proper personal hygiene. Avoid bivalves from contaminated waters.	Cooking	Viruses can not multiply in food.
Seafood	Bacteria	*C. botulinum* type E	Seafood, meat from marine mammals, fresh water fish	Avoid certain traditional fermented foods (eg, mukluk) and fish dried with intestines intact. Consumer education.	Apply thermal process sufficient to kill spores (eg, canning of seafood).	Safety depends on preventing growth to levels sufficient for toxin production. Apply combinations of temperature, aw, pH and time that inhibit growth(eg, temperature < 3C; a_w < 0.97; 3.5% NaCl on water at 5C; or pH below 5.0). Control fermentation conditions to prevent growth and toxin production.
		V. parahaemolyticus	Raw seafood, recontaminated cooked seafood	Avoid uncooked fish where pathogen is prevalent.	Cooking	Avoid recontamination, maintain low storage temperatures.
		V. vulnificus	Oysters	Avoid bivalves from regions of higher risk, avoid wounds to hands when handling crustaceans	Cooking oysters.	Increase of *V.vulnificus* in oysters post-harvest is not a factor.

(continued)

Table 3.1 (continued)

Primary source	Group	Agent	Common vehicles	Control measures		
				Controlling initial level	Reducing level	Preventing increase in level
	Toxin	Scombrotoxin	Tuna, mackarel, mahi mahi	Select freshly harvested and properly chilled fish that are high risk. Specification for histamine; mean of 100 ppm, two of nine samples tested may be >100 but <200 ppm, no sample > 200 ppm	Histamine level can not be reduced after harvest.	Cool fish rapidly and maintain at low temperature (<8 °C; preferably lower) to prevent histamine formation.
		Tetrodotoxin	Pufferfish	Avoid fish from the Tetrodontia family.	Toxin can not be reduced after harvest; toxin is stable.	Toxin can not increase.
		Ciguatera	Certain tropical reef fish	Avoid tropical reef fish from regions and seasons of high risk.	Toxin can not be reduced after harvest; toxin is stable.	Toxin can not increase.
		Shellfish toxins (PSP, DSP, ASP, NSP)	Bivalves	Monitor shellfish harvesting areas for toxic phytoplankton, suspend harvesting if necessary, specification PSP 80 µg/100 g, ASP 20 µg/100 g	Toxin can not be reduced after harvest; toxin is stable.	Toxins can not increase.
	Parasites	*Anisakis*	Herring, cod	Inspect and discard infected fish.	Freezing (−18 °C for 24 h); hold in combination of NaCl and acetic acid; cooking	Parasite can not increase.
Livestock, poultry	Bacteria	Brucellae	Unpasteurised milk and cheeses made therefrom, contact with infected livestock (eg, cattle, goats).	Eradicate brucellosis from domesticated animals. Avoid cheeses made from unpasteurized milk in regions where brucellae are endemic.	Pasteurise milk.	Brucellae do not increase during cheese manufacture or subsequent storage.
		M. bovis	Unpasteurised milk.	Eradicate bovine TB	Pastuerise milk	*M. bovis* can not increase during storage of milk or milk containing foods.
		C. jejuni / coli	Raw or undercooked poultry, unpasteurised milk.	Effective on-farm and slaughtering control measures for raw poultry and meat are uncertain. Education of food handlers.	Cooking; pasteurise milk	*C. jejuni / coli* are not likely to multiply in food due to the high temperature (≥32C) and other requirements for growth. Cross contamination to ready-to-eat foods is a significant concern.

		Salmonellae, non-typhoid	Wide variety of foods.	Select foods and ingredients from suppliers with effective control systems. Understanding the limitations of current control measures to prevent or eliminate salmonellae from raw agricultural commodities.	Cooking; pasteurisation; acidification; other combinations (eg, a_w, pH, temperature, preservatives); irradiation.	Use of inhibitory factors (preservatives, a_w, acidifiers, low temperature storage) alone, or in combination, to inhibit growth. Minimize cross contamination.
		Pathogenic *E.coli*	Undercooked ground beef, raw produce, unpasteurized juice, sprouts, untreated water, contact with ruminants	Effective on-farm control measures have not been identified. Control measures during slaughter can reduce but not eliminate these pathogens. Effective GAPs (good agricultural practices) for crops have yet to be demonstrated. Selecting raw agricultural commodities from suppliers with a system of comprehensive control measures can minimize but not prevent the presence of these pathogens. Avoid untreated water and contact with livestock.	Cooking; pasteurizing; washing and disinfecting (eg, fruits, vegetables).	Multiplication can occur in many foods but this has not been a significant factor. Survival and low infective dose among the more sensitive populations are much more important.
		Y. enterocolitica	Raw or undercooked pork, untreated water, milk	Improved slaughter hygiene (ie, sealing off rectum in plastic bag).	Cooking	Minimize contact of ready-to-eat foods with raw pork
		Staph. aureus	Cooked foods contaminated with Staph. aureus and held at conditions permitting growth and enterotoxin production. *Staph. aureus* does not compete well against the normal flora associated with raw agricultural commodities and, thus, these foods are rarely involved.	Selecting foods and ingredients that are likely to have lower initial numbers. Preventing food handlers with boils, infected cuts from handling food.	Cooking, pasteurizing, acidifying, etc. can reduce the number of Staph. aureus in food. Staphyloccocal enterotoxin is very stable and can not be reduced by normal methods of food preparation.	Control temperature and time of holding cooked perishable foods to prevent sufficient growth for enterotoxin production. Use of inhibitory factors (eg, a_w, preservatives, acidifiers, smoke) to inhibit growth. Control conditions for manufacture of certain cheeses and dry sausages where growth during fermentation and, in the case of cheese, pressing is important.
	Parasites	*Taenia*	Raw or undercooked pork and beef	Break epidemiological chain at final host (man) or intermediate host (cattle, pigs); animal husbandry; treat sewage and sludge; meat inspection	Cook to >60 °C; freeze to −10° 6 days	This parasite can not increase during processing or storage.
		Trichinella spiralis	Raw or undercooked pork, wild game, horse meat.	Select pigs from sources (eg, industrialized farms) and regions of little or no risk; eradicate infested pigs and manage the risk of consumer exposure to infested wild game; avoid feeding uncooked kitchen waste to pigs; adopt meat inspection procedures to eliminate infested carcasses.	Freeze −25 °C for 10–20 days; cook to 58 °C minimum.	*T. spiralis* can not increase in food.

(continued)

Table 3.1 (continued)

Primary source	Group	Agent	Common vehicles	Control measures		
				Controlling initial level	Reducing level	Preventing increase in level
Soil	Bacteria	*L.monocytogenes* (Sources include the soil, environment of food establishments and homes, raw agricultural commodities, animals – including humans)	Perishable ready-to-eat foods in which growth can occur (eg, certain soft cheeses, milk, cooked meats and poultry, smoked seafood)	Select foods subjected to a kill step, where possible; control the ready-to-eat environment to minimize contamination; education of higher risk consumers of foods to avoid.	Cooking; pasteurize milk; pasteurise in the container in which a food will be sold; formulating foods to create listericidal conditions during subsequent processing, storage and distribution (eg, acidification, dehydration); washing and disinfecting.	Formulating foods with additives or creating conditions that can prevent multiplication. Storing perishable foods at low temperatures that can retard or prevent growth. Manage the environmental conditions of the operation to minimize the likelihood of contamination from raw foods/ ingredients or contaminated surfaces. Use controlled fermentations that prevent growth.
		B. cereus	Rice, cereal products, certain dairy products, sauces, cooked pasta, vegetable or meat dishes.	Control measures can not ensure the absence of this pathogen in the foods commonly involved.	Cooking can kill the vegetative cells but spores may survive.	Cool rapidly from 50C to 10C after heating; store below 10 °C or above 60 °C;
		C. botulinum type B (non-proteolytic)	Dry cured ham and other salt cured meats and seafood.	Control measures can not ensure the absence of this pathogen in the foods commonly involved.	Apply thermal process sufficient to kill spores (eg, canning)	Safety depends on preventing growth to levels sufficient for toxin production. Apply combinations of temperature, aw, pH and time that inhibit growth (eg, temperature < 3C; a_w < 0.97; 3.5% NaCl on water at 5C; or pH below 5.0). Control fermentation conditions to prevent growth and toxin production.
		C. botulinum type A, B (proteolytic)	Low acid foods, meat, home canned vegetables and low acid fruits.	Control measures can not ensure the absence of this pathogen in the foods commonly involved. Consumer education to avoid tasting suspect food.	Apply thermal process sufficient to kill spores (eg, canning)	Safety depends on preventing growth to levels sufficient for toxin production. Apply combinations of temperature, aw, pH and time, etc. that inhibit growth (eg, temperature < 10C; a_w < 0.94; 10% NaCl on water; pH below 4.6); sodium nitrite in cured meats.
		C. perfringens	Cooked uncured meat or poultry, meat pie, stew, gravy	Control measures can not ensure the absence of this pathogen in the foods commonly involved.	Cooking to kill vegetative cells, retorting to kill spores. Storage at low temperatures can cause significant decrease in vegetative cells.	Cool rapidly from 51C to 15 °C after cooking; store below 10–12C or above 51C, preferably at or above 60C.

		A.flavus, A. parasiticus	Corn, peanuts	Monitor weather and test crops when conditions indicate higher risk. Minimize insect infestation. Reject moldy products.	The mycotoxins are very stable.	Rapid drying and dry storage to prevent growth of fungi and toxin production.
		A. ochraceus	Beans, dried fruit, nuts, cereals, biltong, dried fish	Monitoring weather and testing crops when conditions indicate higher risk. Reject moldy products.	Toxins are stable	Rapid drying and dry storage to prevent growth of fungi and toxin production.
		A. versicolor	Wheat, corn, flour, rice, nuts, spices, fermented meats, cured meats, biltong, cheese	Prompt harvest when ready. Reject or test suspect foods for the toxin.	Toxin is stable	Rapid drying and dry storage to prevent growth of fungi and toxin production.
		Fusarium graminearum	Wheat	Prompt harvest when ready. Reject or test suspect lots for the toxin.	Toxins are stable	Rapid drying and dry storage to prevent growth of fungi and toxin production.
		Fusarium moniliforme	Rice, sorghum, sugar cane, corn	Prompt harvest when ready. Reject or test suspect foods for the toxin.	Toxins are stable	Rapid drying and dry storage to prevent growth of fungi and toxin production.
		Fusarium sporotrichoides	Cereals, peanuts, soya beans	Prompt harvest when ready. Reject or test suspect foods for the toxin.	Toxins are stable	Rapid drying and dry storage to prevent growth of fungi and toxin production.
		Penicillium citrinum, *P.crustosum*, *P. islandicum*, *P.verrucosum*	Ubiquitous, especially rice, wheat and corn	Prompt harvest when ready. Reject or test suspect foods for the toxin.	Toxins are stable	Rapid drying and dry storage to prevent growth of fungi and toxin production.

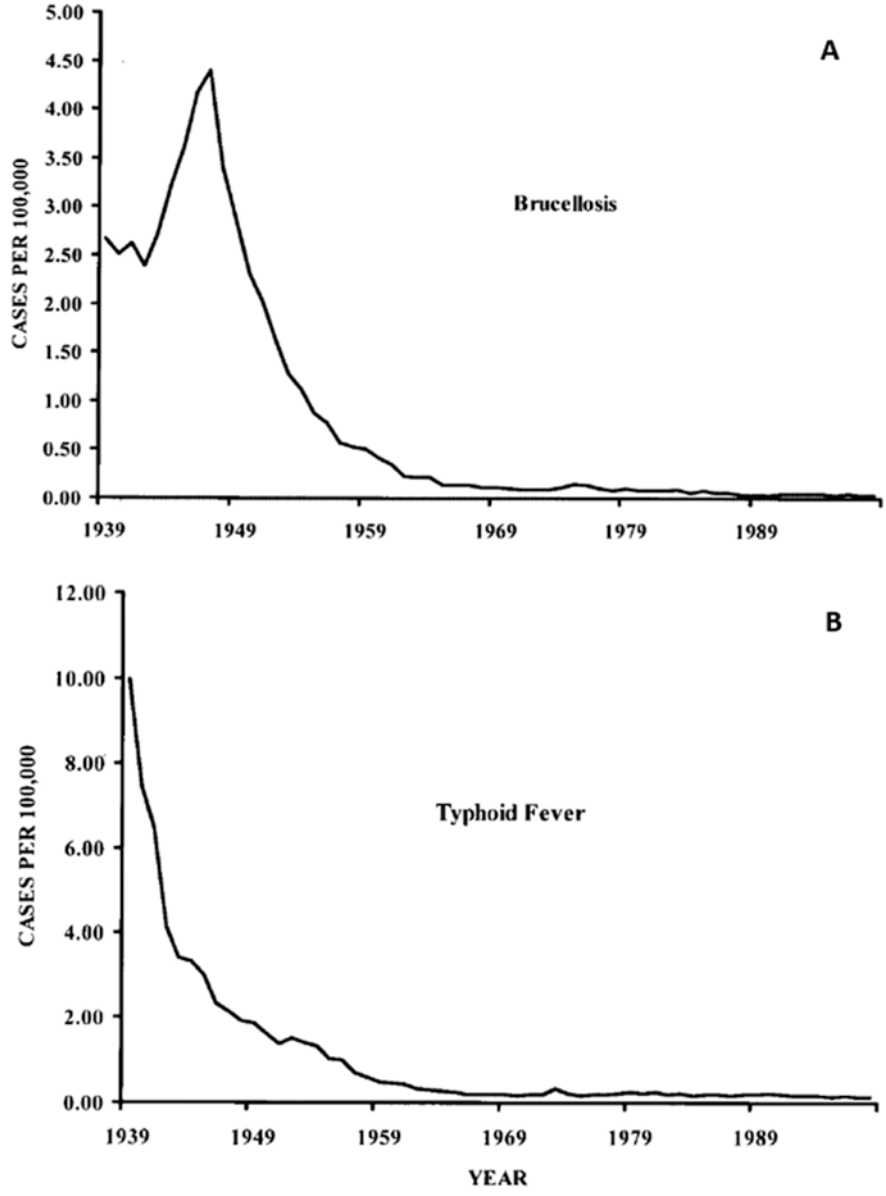


Fig. 3.2 Reduction of (**a**) trichinosis and (**b**) typhoid fever in the USA after World War II

been used to detect and eliminate carcasses with *T. spiralis* (Pozio 1998). The different approaches have led to differences in regulatory requirements, quality characteristics of pork products and culinary habits. In this particular example, however, the European approach was much more effective and achieved more rapid reductions in the prevalence of *T. spiralis* in the hog population and human trichinosis than in the USA. It is only in recent years that the USA prevalence of human trichinosis has begun to approach the levels found in Europe before 1940. In the EU, 74.4% of domestic pigs are raised on industrialized farms where *Trichinella* infection has not been diagnosed. The reservoir for *Trichinella* in the EU consists of pigs raised on small family farms or grazing in wild areas, wild boars

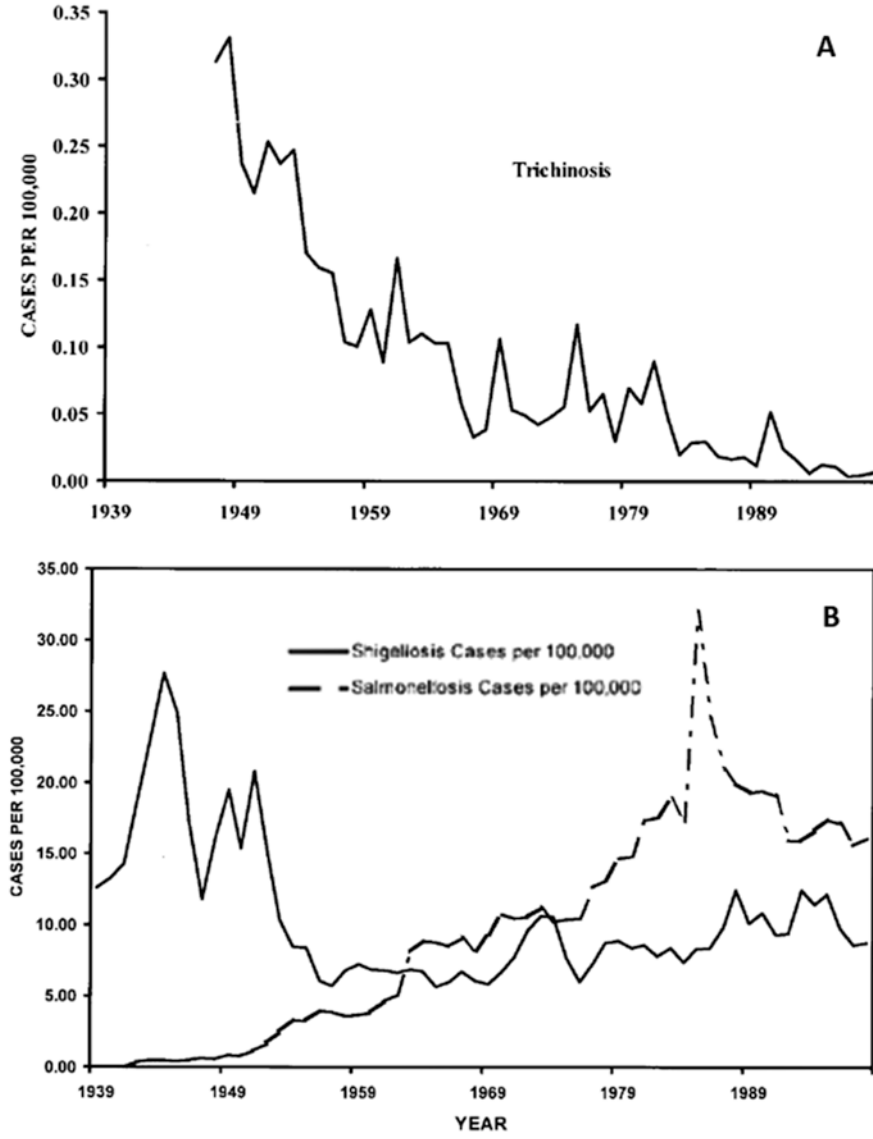


Fig. 3.3 Cases of (**a**) human trichinosis and of (**b**) shigellosis and salmonellosis, per 100,000 population in the USA

and horses (Pozio 1998). The prevalence of trichinellae in pigs in Germany has been three or fewer infected pigs among about 40 million pigs slaughtered each year (Rehmet et al. 1999). In a review of trichinellosis in The Netherlands it was stated that such infections had not occurred in humans or animals during the previous 40 years (Ruitenberg and Sluiters 1974). For comparison, farm raised swine, which comprised 98.5% of the swine production in the USA, had prevalence rates of about 0.125% (Zimmerman 1974).

The third example, shown in Fig. 3.2b, is the sharp decline in typhoid fever in the USA from 1939 through 1998 (CDC 2000a). The source of *Salmonella* Typhi is the intestinal tract of humans. Growth outside the human host has not been an important factor. The primary routes of infection have been fecal-oral routes involving untreated water and infected food handlers or health care providers.

Table 3.2 Processes that have been used for making commercially prepared foods shelf-stable (Tompkin and Kueper 1982)

Food	Process code	Food	Process code
Milk	D, H	Vegetables	A, B, H
Eggs	H	Vegetable juices	B, D
Butter	–	Vegetables, pickled	A
Natural cheese	J	Peanut butter	I
Processed cheese and cheese sauce	C	Ham, bacon	A, E
Cake, bread	K, L	Beef	B, E
Cookies, crackers	H	Pork sausage	E, G
Fruit	A, C, J, H	Dry and semi-dry sausage	E, F, G
Fruit juices	C, D	Franks, viennas, meat spreads	B
Jams, jellies	C	Luncheon meats, cured	A
		Fish, smoked salmon, pickled herring, salt cod	B, E, H

Process Codes:

A. Mild thermal process ($F_0 = < 2.78$) in hermetically sealed container.
B. High thermal process ($F_0 = \geq 2.78$) in hermetically sealed container.
C. Thermal process, hot fill, seal and hold before cooling
D. Thermally process, chill, then aseptically package.
E. Salted, perhaps cured, at low temperature then dried during heating or at ambient temperatures. Smoke commonly applied.
F. Fermented at 7.2, perhaps heated to ≥46 °C, then dried at cool temperature (e.g., 13 °C).
G. Fermented and/or cooked, dried, then sealed in lard.
H. Thermal process and dehydration.
I. Roasted, ground and filled at moderate temperature.
J. Dehydration
K. Filled into container, sealed, then heated sufficiently to bake product.
L. Baked in container, then sealed while hot.

Table 3.3 Maximum allowable frequencies for salmonellae on carcasses and ground meat and poultry (USDA 1996)

	Allowable percent positive samples	Number of Samples	Maximum allowable number of positives
Carcasses:			
Steer/heifer	1.0%	82	1
Cows/bulls	2.7%	58	2
Hogs	8.7%	55	6
Broilers	20.0%	51	12
Ground products:			
Beef	7.5%	53	5
Chicken	44.6%	53	26
Turkey	49.9%	53	29

Thus, improvements in waste water management; availability of safe, potable water for drinking and food processing; and education on the importance of hand washing have led to reductions in the prevalence of typhoid fever. This enabled a more directed approach toward identifying and treating individuals who harbored the pathogen due to infection or as asymptomatic carriers. Finally, vaccination of individuals travelling to regions where typhoid fever was endemic reduced the likelihood of re-introducing the pathogen into the population.Figure 3.3b reports the trends for shigellae and non-typhoid salmonellae in the USA over a period of 60 years (CDC 2000a). While progress was initially made toward reducing the rate of shigellosis, no further improvement seemed to be made towards the

end of the time period reported on. Shigellae are similar to *S.* Typhi in that humans are the source and transmission involves contaminated water and food. An additional significant route involves people-to-people transmission via the fecal-oral route, particularly in institutional settings (e.g., child care centers). At the time of issuing the report, CDC considered that when effective control measures could be implemented these would decrease the prevalence of shigellosis below the level of 5–8.9 cases per 100,000 (CDC 2000a). Please note that Chap. 2, Fig. 2.1 shows the reported incidence of these illnesses from 1980 to 2013, which show shigellosis trending down towards five cases per 100,000.The data for non-typhoid salmonellae show an increase of salmonellosis until about 1990, followed by a decrease (Fig. 3.3b). The decrease may be attributed to reductions in egg borne transmission of *S.* Enteritidis through improved on-farm controls (CDC 2000b). In addition, some reduction may eventually prove to be due to improved controls implemented by the meat and poultry industry following the establishment of performance standards by the USDA-FSIS in 1996 (USDA 1996). As is apparent from Chap. 2, Fig. 2.1, the level of salmonellosis cases remained in the range of 15–20 cases per 100,000 towards the year 2010.

Concerning the USDA-FSIS performance standards noted above, details are given in Table 3.3. In effect, these performance standards could be considered "implicit" or "default" POs because they specify maximum frequencies for salmonellae in specific food categories tolerated at particular stages in the food supply chain, i.e. on carcasses and in ground meat. Because these performance standards have been incorporated into a regulation, they are mandatory regulatory requirements, although they are different from microbiological criteria that can be used as standards as described in Chap. 5.

In the section on the feasibility of meeting FSO/PO values, the need for industry investing resources was discussed as one of the options when current resources would be insufficient. In this regard, it is of note that, in anticipation of the USDA-FSIS regulation and other requirements, the meat and poultry industry in the USA invested extensively to modify its processing equipment, plant layouts and processing procedures. This included the use of antimicrobial treatments such as improved control of chlorinated processing water for poultry and the use of steam or hot water pasteurization for beef carcasses. Some modifications were implemented well in advance of the effective dates for the regulatory requirements.

All categories have shown reductions in the prevalence of salmonellae in large plants from 1996 to 2000. The prevalence rate for broilers in large plants, for example, declined to approximately 10% (USDA 2000). Thus, it would appear that the performance standards have led to reductions in the prevalence of salmonellae while allowing industry to decide how to modify its processing conditions. The question remains whether the reductions in salmonellae in raw meat and poultry will lead to a measurable reduction in human salmonellosis in the US. In addition, it is uncertain whether further reductions will be possible in the US in absence of new technology or improved control at the farm level. In Finland, Norway, and Sweden greater emphasis has been placed on testing and control at the farm level to achieve on-farm prevalence rates of less than 1% for salmonellae in livestock and poultry. These control measures were intended to meet a performance standard of less than 1% prevalence rate for salmonellae in foods of animal origin (Kruse 1999). In Europe, the confirmed case rate for *Salmonella* infection was still relatively high (20.4 per 100,000 population in 2011), although the incidence of salmonellosis has been declining steadily since 2004 as a result of the implementation of successful veterinary control programs, particularly in poultry (ECDC 2013).

A final example of an effective control measure has been the apparent 40% reduction in human yersiniosis in Norway following a rather simple but effective change in the slaughtering procedure for hogs. Data indicate the reduction occurred after about 90% of the hog slaughter-houses implemented a procedure of sealing off the rectum with a plastic bag immediately after it was cut free. A similar decline (about 30%) reportedly occurred following adoption of the same practice in Sweden (Nesbakken 2000).

3.5 Deriving Performance Criteria

To achieve the defined FSO or PO it is generally necessary to implement control measures at one or more different steps in the food supply chain as part of the implemented food safety management system. At these steps, the outcomes in terms of level of hazards can either be actively controlled, for instance by applying control measures that limit or reduce the prevalence and/or concentration of a hazard. However, in some situations, active control may not be applied and hazards may then be allowed to increase to the extent that conditions and the food matrix support their growth. In any case, the dynamics of a hazard within a step as well as in subsequent steps of the food supply chain (or value chain) need to be thoroughly understood to assure the safety of a food product at the point of consumption.

In principle, each step in the value chain will see a hazard coming in at a particular level (designated the incoming hazard level or H_0) and a food product or material leaving the step with a particular maximum hazard level (the PO or FSO). The control measures implemented in the food safety management system of a step will influence the change, if any, between H_0 and PO/FSO.

When establishing a safe product and process design and implementing this in the food safety management system of a step, control measures may be chosen in such a way that the change in hazard level across the particular step is being actively controlled.

The required outcome of the (set of) control measures implemented in the food safety management system across a particular step in the value chain is referred to by Codex as the *Performance Criterion* (PC). Codex defines the PC as: *the effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a PO or an FSO* (CAC 2015).

Since a PC articulates an outcome that should be achieved by a control measure or a series or a combination of control measures, the PC forms the actual bridge between traditional food safety metrics (i.e. Microbiological Criterion/MC, Process Criterion/PcC, Product Criterion/PdC) and the expected level of public health protection, first being defined at population level (e.g. ALOP) and converted to supply chain level expectations (FSO/PO). Figure 1.1 in Chap. 1 illustrates this.

A PC may be used in conjunction with a microbiocidal (e.g., thermal treatment, antimicrobial rinse) or microbiostatic (e.g., refrigeration, water activity reduction) control measure. CAC (2007a) suggests that a PC for a microbiocidal control measure could also express the desired reduction of the microbial population that occurs during the application of the control measure (e.g., 5-log reduction in the levels of *L. monocytogenes*). A PC for a microbiostatic control measure could express the maximum increase in the microbial population that is acceptable under the various conditions during which the measure is applied (e.g., less than a 1-log increase in *L. monocytogenes* during refrigerated distribution of a ready-to-eat food).

While ALOP and FSO would only be the purview of competent authorities, PCs as well as POs are generally set by individual food business operators. However, competent authorities may also establish POs and/or PCs where they wish to provide advice to food businesses that they believe are not capable of establishing POs/PCs themselves, but where they are of critical importance to the overall performance of the particular food supply chains. There are several factors that have to be considered in reaching a decision on the value of a PC such as, amongst others, the variability of pathogen levels in raw ingredients or the variability associated with a processing technology. Where competent authorities stipulate PCs to encourage the affected food business operators to adopt specific control measures, the application of these by industry should generally be uniform.

Such PCs are often translated by industry or sometimes by competent authorities into a PcC or a PdC (CAC 2007a). For example, if a PC indicated that a heat treatment should provide a 5-log reduction of a hazard, then the corresponding process criteria would stipulate the specific time and temperature combination(s) that would be needed to achieve the PC. Similarly, if a PC required that an

acidification treatment of a food reduce the rate of growth of a hazard to less than 1-log in 2 weeks, then the product criterion would be the specific acid concentration and pH that would be needed to achieve the PC. The concepts of process criteria and product criteria have been long recognized and used by industry and competent authorities.

According to CAC (2007a), in many instances, a PC describes the outcome that is needed in order to achieve a PO at a specified point in the food chain. Consequently, a PC could cover the entire step in a food supply chain that is managed by an individual food business operator such that the incoming hazard level for the step is managed to the maximum hazard level targeted for the material leaving the step (i.e. PO/FSO, when defined). Note that a step would be an operation for which a specific food safety management system has been put in place by the responsible business operator, such as primary production, slaughter, storage, distribution, manufacturing, retail, or food service.

For PCs covering a step in the value chain step, generally, several different control measures are applied in concert to achieve a particular PC, e.g. cold storage of ingredients, heat processing, rapid cooling, physical separation of raw and processed materials, and packaging. The rationale is that various control measures provide relevant contributions to the PC that cannot be met by a single measure, for instance by controlling growth of hazards, reducing hazards to target levels, or avoiding recontamination after a key reduction step. Conceivably, when a very large reduction in hazard levels through pasteurization or sterilization would not be followed by measures that control recontamination up to the point of packaging, the level of hazard in the finished product would possibly not be under control and the PO/FSO might not be met.

Please note that, following the guidelines established by Codex Alimentarius (CAC 2007a), performance standards such as the following could qualify as PCs:

- 12D reduction of proteolytic *Clostridium botulinum* in low acid canned foods (Stumbo 1973; Brown 1997).
- 6D reduction of *Listeria monocytogenes* in ready-to-eat chilled foods (Lund et al. 1989).
- 6D reduction of psychrotrophic strains of *Clostridium botulinum* in pre-prepared chill-stored foods with extended shelf life (ACMSF 1992; Gould 1999).
- 5D reduction of *Escherichia coli* O157:H7 for fermented meat products (Nickelson et al. 1996) and fruit/vegetable juice (FDA 2001).
- 6.5D for salmonellae in cooked beef (CFR 2008a) and 7D for salmonellae in cooked poultry (CFR 2008b).

To achieve the reductions stipulated by these performance standards, operators may select specific equipment and process criteria (i.e. time and temperature settings for the equipment) most suitable for their operation and product.

In practice, additional control measures (e.g. avoidance of recontamination after heat treatment or at packaging; refrigeration during shelf-life) are often needed to complement such hazard reduction steps in a concerted fashion in order to achieve the targeted hazard level in the material or product leaving the step.

In this regard, it would be possible to refer to the required overall control of a hazard in a step as the PC, which is operationalized by establishing the necessary individual control measures to for instance limit growth (e.g. a product criterion), reduce a hazard (e.g. a process criterion), and/or prevent cross-contamination (e.g. aseptic packaging, zoning) such that a PO/FSO is met.

When establishing PC values, consideration must be given to the initial level of a hazard, which in the following is designated as the "H_0", and changes required therein during the food supply chain step at hand, in order to deliver the PO (for points upstream of consumption) or the FSO (when the PC relates to the point of consumption).

In the course of a step, there may be events or opportunities that lead to increases or reductions in the level (i.e. concentration, frequency or both) of a hazard in a food or in component parts ending up

in the finished product. In some cases, single events may lead to increases or reductions (e.g. mixing or dilution, applying a non-lethal or lethal heat treatment, etc.), but there can also be several different events at time points within an operation that have such effects. All these may impact on the actual dynamics of a hazard as food materials moves through a step and need to be understood and managed to assure that a safe food material/product leaves the step. In all cases, the PC value required across a particular step will be determined by the sum of increases, designated as "ΣI", and the sum of decreases, designated as "ΣR".

In the first edition of the current book (ICMSF 2002), ICMSF proposed the following *conceptual equation* to, at a relative high level of granularity, derive the PC for a step from the incoming hazard level (H_0) such that the outcome target (PO or FSO, depending on the stage in the value chain) is delivered:

$$H_0 - \Sigma R + \Sigma I \leq \text{FSO or PO} \tag{3.1}$$

Where:

FSO = Food Safety Objective
PO = Performance Objective
H_0 = Initial level of the hazard
ΣR = Total (cumulative) reduction of the hazard
ΣI = Total (cumulative) increase of the hazard

FSO, H_0, R and I are all expressed in $\log_{10}$ units. By definition, the value of R will introduce a reduction in the calculated hazard level and I an increase.

From this conceptual equation it follows that PC = − ΣR+ΣI (Eq. 3.2), when the PC is considered to be the overall required level of control over a hazard in a step, from the incoming hazard level to the outcoming hazard level.

Please note that the *conceptual equation* coined by ICMSF is meant as a rough guide to reason through various relevant events influencing hazard levels across a particular step of the food supply chain upward or downward at a relatively high level of granularity. The equation is not meant as an accurate mathematical reflection of reality and may not account for the specific events that may occur within a particular step in all possible situations. In this regard, it is a simplification of reality and should first of all be considered as a starter of the thought process for establishing a safe product and process design and setting up an adequate food safety management system [in an operation for a particular product].

Note that this equation by default assumes 100% prevalence [/frequency], i.e. assumes a particular [absolute or mean] concentration of a hazard occurs across every portion of a food material/batch, but can be used in conjunction with a known or estimated prevalence (Zwietering 2005).

In the text below, examples are provided of the utility of the conceptual equation across a step in the value chain in a number of situations and at a high level of granularity with a focus on changes in levels of contaminants due to growth (i.e. Examples 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, and 3.9). Examples 3.8, 3.9, and 3.10 illustrate situations in which both growth as well as recontamination play a role and where particular attention needs to be given to the utility of the equation to more properly account for the additive effects on both log and linear scale. Finally, Example 3.11 reflects on other situations where additions or reductions may occur on a linear scale due to discrete events. For simplicity, no difference is made in these examples between meeting either a PO or FSO considering that the same principles apply for establishing a safe product design and an appropriate food safety management system albeit at different stages/steps in the value chain.

Guidance will be given on utility of the equation in general terms (Sect. 3.5.1) as well as for specific cases (Sect. 3.5.2) for which a more granular insight in hazard dynamics within a step requires

tailored approaches. However, even at the high level that the equation may be used across a step, in many instances, it may support decisions related to the design of an appropriate food safety management system at that step and assist in evaluating candidate control measures that may be able to achieve a desired outcome target such as a specified PO/FSO. Obviously, the suitability of candidate control measures needs to be duly validated at the appropriate scale of operation.

3.5.1 Examples of General Use of the ICMSF Conceptual Equation

In the following three examples, illustrated in (Figs. 3.4, 3.5, and 3.6), the same hypothetical dose-response curve for a certain infectious pathogen is considered (shown in the top of each figure), as a result of which the estimated number of cases per 100,000 population increases with pathogen concentration. In all three examples, an FSO has been established at 1 cfu/100 g or -2 $\log_{10}$ cfu/g (note that a PO could be set to this level earlier in the value chain).

Example 3.1

In this example (Fig. 3.4**),** the maximum initial population (H_0) in the raw material entering a step is estimated to be 10^3 cfu/g. Both growth from the initial population and contamination from other sources can be completely prevented, due no increase in the hazard level during the step ($\Sigma I = 0$). The PC required across the step to deliver the PO/FSO for the step can then be derived from Eqs. (3.1) and (3.2) as follows:

$$\begin{aligned} &H_0 - \Sigma R + \Sigma I \le \text{FSO or PO}(1) \text{ and PC} = -\Sigma R + \Sigma I \\ &3 - \Sigma R + 0 \le -2, \text{in which} 5 \le \Sigma R, \text{thus} \\ &\text{PC} = -5 + 0 \ge -5 \end{aligned} \tag{3.2}$$

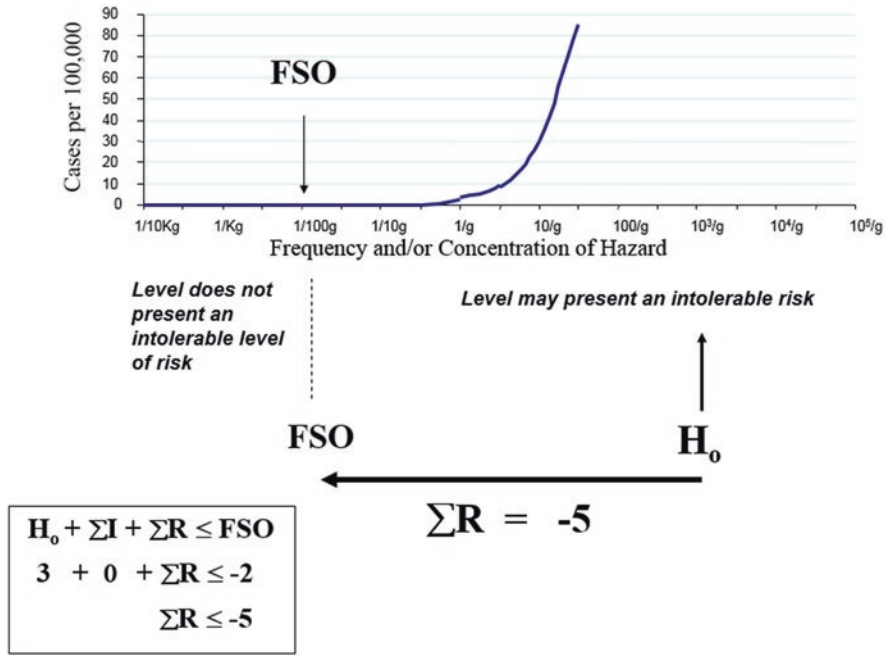


Fig. 3.4 Example 3.1 (see text)

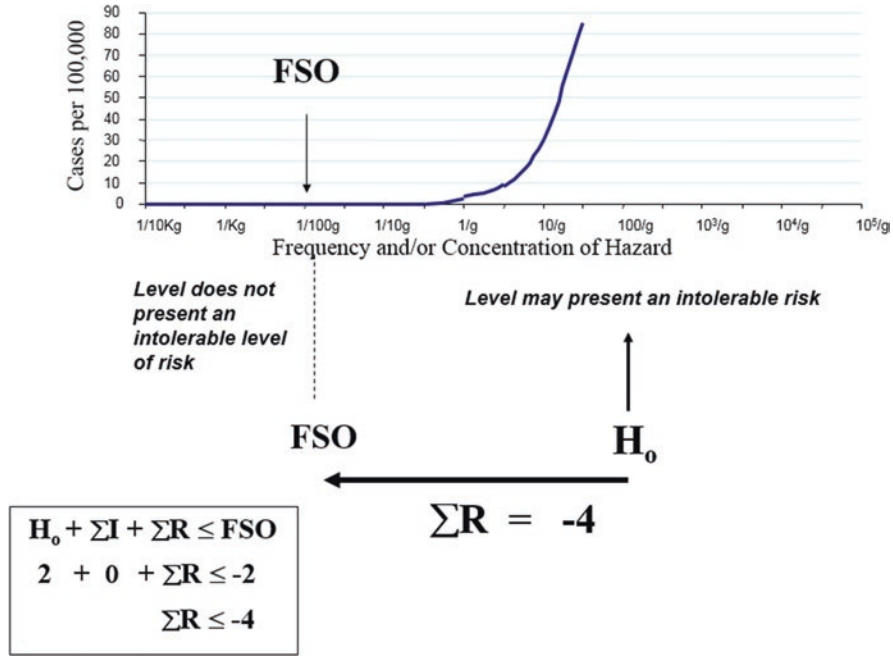


Fig. 3.5 Example 3.2 (see text)

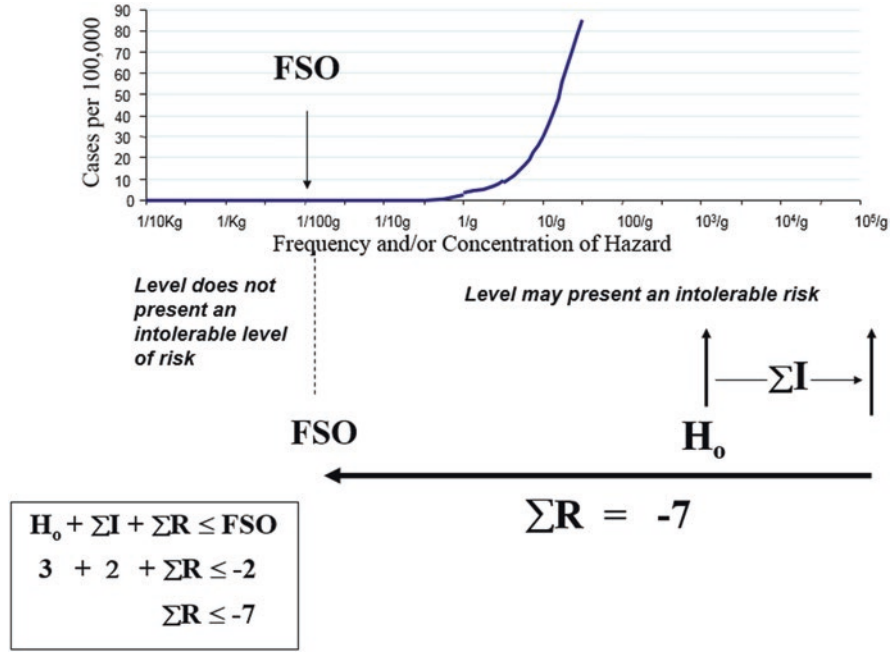


Fig. 3.6 Example 3.3 (see text)

This means that across this particular step, the food safety management system to be implemented should achieve an overall reduction in initial hazard level of 5 $\log_{10}$ or more in order to meet the FSO/PO. This corresponds for instance to a performance standard that delivers a 5D reduction in the level of the pathogen in combination with control measures that completely avoid re- or cross-contamination from happening after the 5D reduction treatment.

Importantly, the food safety management system of the operator of the previous step that supplies the incoming material to the current step needs to assure that the outcoming hazard level (i.e. the PO of the previous step) is no higher than 1000 cfu/g. Should the hazard level of the previous step, which represents the H_0 of the current step, be higher than accounted for, the calculated PC will not achieve the FSO/PO.

A suitable microbiological criterion may be established to verify that an incoming hazard level (H_0) and outcoming hazard level (PO/FSO) meets the level targeted at (Zwietering et al. 2015). Note that comparable supplier requirements and verification tools for H_0 and PO/FSO apply to all examples following.

Example 3.2

In this example (Fig. 3.5)**,** the maximum initial population (H_0) in the raw material can be controlled to ≤100 cfu/g and any increase in hazard level during the step (ΣI) can be prevented completely. The required PC follows from:

$$\begin{aligned} &H_0 - \Sigma R + \Sigma I \le \text{FSO or PO}(1) \text{and PC} = -\Sigma R + \Sigma I \\ &2 - \Sigma R + 0 \le -2, \text{in which } 4 \le \Sigma R, \text{thus} \\ &\mathbf{PC} = -4 + 0 \ge -4 \end{aligned} \tag{3.2}$$

In this case, the management system should achieve an overall reduction of at least 4 $\log_{10}$ units to meet the FSO/PO of −2 $\log_{10}$, corresponding to for instance a performance standard that delivers a 4D reduction in hazard level combined with control measures that avoid any subsequent contamination.

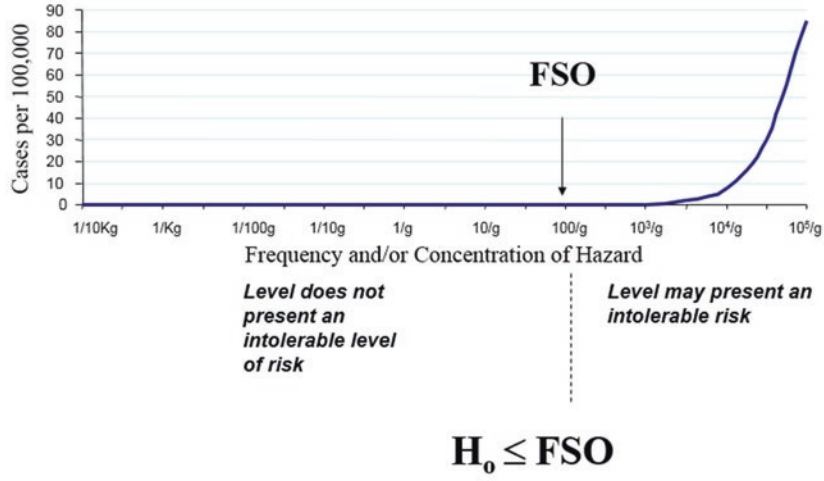
Example 3.3

In this example (Fig. 3.6), the initial population (H_0) in the raw material at maximum is 10^3 cfu/g, but also growth of the hazard is possible within the step concerned. However, control measures are in place to limit the increase of the hazard due to growth to 100-fold (2 $\log_{10}$) and to avoid contamination. The PC required across the step would be deduced as follows:

$$\begin{aligned} &H_0 - \Sigma R + \Sigma I \le \text{FSO or PO}(1) \text{and PC} = -\Sigma R + \Sigma I \\ &3 - \Sigma R + 2 \le -2, \text{in which } 7 \le \Sigma R, \text{thus} \\ &\mathbf{PC} = -7 + 0 \ge -7 \end{aligned} \tag{3.2}$$

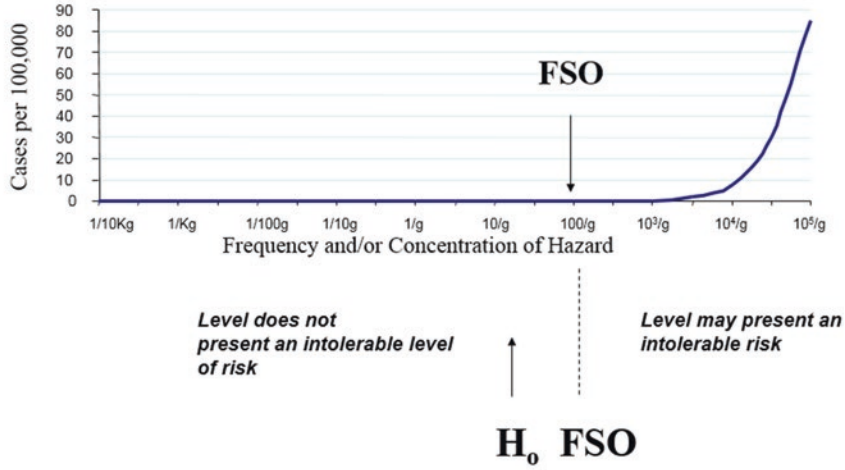
In this example, the management system should achieve at least an overall reduction of 7 $\log_{10}$ units (i.e. from 10^5 cfu/g to ≤1 cfu/100 g) to meet the FSO/PO. This could for instance be achieved by heat processing targeting a performance standard of 7D reduction in combination with a control measure that can limit growth to a maximum of 2 $\log_{10}$ units (e.g. cooling during storage within the step for a time limited period) plus a control measure for complete avoidance of post-process contamination (e.g. aseptic packaging).

Examples 3.4, 3.5, 3.6, and 3.7 (Figs. 3.7, 3.8, 3.9, and 3.10) provide additional cases of the use of the conceptual equation for situations where the increase in hazard level through recontamination and/or cross-contamination does not play a significant role. Examples 3.8, 3.9, and 3.10 relate to situations where contamination from external sources or discrete events require tailored approaches to correctly quantify the changes in hazard levels, because these following with involve additions on the non-logarithmic scale in contrast to for instance growth and inactivation.



$$H_0 + \sum I + \sum R \le FSO$$
$$H_0 + 0 + 0 \le 2$$
$$H_0 \le 2$$

Fig. 3.7 Example 3.4 (see text)



$$H_0 + \sum I + \sum R \le FSO$$
$$1 + \sum I + 0 \le 2$$
$$\sum I \le 1$$

Fig. 3.8 Example 3.5 (see text)

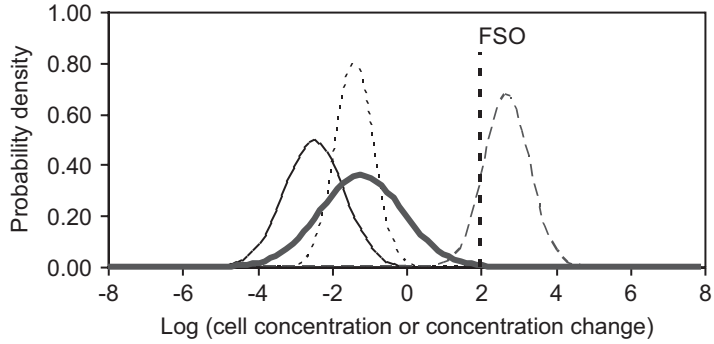


Fig. 3.9 Probability distribution of initial cell level (H_0 ___), reduction in concentration (ΣR– – –) and increase in concentration (ΣI– – –) of *Listeria monocytogenes* on fresh cut lettuce, and resulting cell concentration distribution (←) in packages of lettuce at the point of consumption using input values in Table 2.2

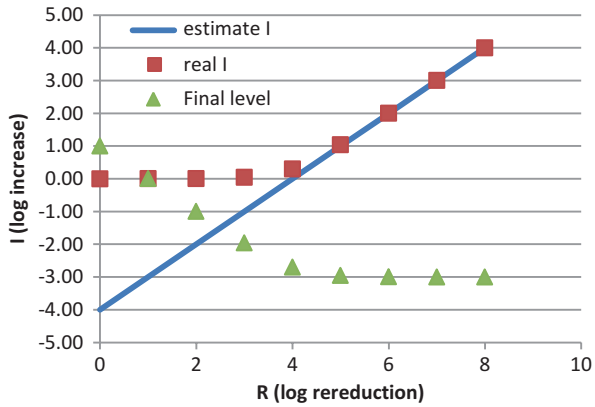


Fig. 3.10 Impact of level of inactivation between 0 and 8D on final hazard level, for a 1000 kg batch of food with a pre-processing hazard level of 1 log/g and post-processing contamination with 1 g of material containing 3 $\log_{10}$/g of the hazard

Example 3.4

In this example (Fig. 3.7), there is no reduction event possible within the step concerned, i.e. no control measure available to reduce the incoming hazard level or to offset an increase within the step due to growth or contamination. Since there is no change in the hazard level possible within the step, the incoming hazard level should not exceed the FSO/PO set for product leaving the step:

$$\begin{aligned} &H_0 - \Sigma R + \Sigma I \le \text{FSO} \quad \text{or PO}(1) \text{and PC} = -\Sigma R + \Sigma I \\ &H_0 - 0 + 0 \le -2, \text{in which PC} = 0 + 0 = 0, \text{thus} \\ &H_0 \le -2 \end{aligned} \tag{3.2}$$

In this scenario, the pathogen level coming into the step should be equal or lower to 1 cfu/100 g in order to meet the FSO/PO set as the outcome for this step. To achieve this, appropriate control measures need to be applied in the previous step (i.e. the suppliers' operations).

Additionally, any increase in hazard level within the step concerned needs to be avoided by applying appropriate growth controlling measures, such as intrinsic or extrinsic factors, in combination with measures to prevent contamination from external source, such as applying laminar flow or packaging. Should it not be possible to completely control an increase in hazard level across the step concerned, the H_0 should be managed proportionally lower by the supplier/operator responsible for the previous step and adequate verification in place.

Example 3.5
In this example (Fig. 3.8), the initial level of the pathogen can be controlled to ≤10 cfu/g and any form of contamination can be confidently prevented. However, growth may be possible in the course of the step and a PC needs to be determined that sufficiently limits such growth. Assuming that the PO or FSO has been set at 100 cfu/g (2 $\log_{10}$), the required PC can be deduced as follows:

$$\begin{aligned} &H_0 - \Sigma R + \Sigma I \le \text{FSO or PO}(1) \text{ and PC} = -\Sigma R + \Sigma I \\ &1 - 0 + \Sigma I \le 2, \text{in which } 1 \le \Sigma I, \text{thus} \\ &\text{PC} = 0 + 1 \ge 1 \end{aligned} \tag{3.2}$$

Thus, to ensure that the FSO/PO is met, the increase in pathogen concentration across the step due to growth must not be more than ten-fold. This may be achieved through the establishment of appropriate product criteria based on a_w and pH, either alone or in combination. Whether such control measures adequately cap growth, needs to be properly validated. Also, again, assurance is needed from the food business operator(s) or supplier providing the relevant incoming raw material or ingredient(s), that the incoming hazard level (H_0) is no higher than 10 cfu/g, otherwise the PC determined would not be effective to achieve the FSO/PO.

Example 3.6
It should be recognized that in all of the five examples described above, the values for the various parameters of the conceptual equation were chosen as point estimates. However, in practice, each parameter will likely have a distribution of values associated with them that reflects the variability of the particular parameter. If stochastic data (i.e., data on the variability associated with one or more different parameters) are available, then these probability distributions may be used rather than point estimates. Examples 3.6 and 3.7 illustrate the use of stochastic data to develop a risk management framework for the control of E. coli O157:H7 and *Listeria monocytogenes* on in pre-cut lettuce, respectively. Further examples can be found elsewhere (ICMSF 2011; Zwietering et al. 2010).

Example 3.6 is explored in more detail in Chap. 17, where the use of risk metrics is illustrated in establishing control measures for enterohaemorrhagic *Escherichia coli* in leafy vegetables. The example makes use of data collected for initial levels of product contamination, efficacy of processing interventions, and growth during distribution. A public health goal of a 50% reduction in risk, established by a competent authority based on epidemiological data, was translated into a Food Safety Objective (FSO). Taking the FSO as a starting point, the ICMSF equation and the ICMSF risk management tool (ICMSF 2010, 2015a) is used to explore options for food safety risk management of leafy vegetables including specification of a Performance Objective (PO) related to processing. In this example, the ICMSF equation is used to demonstrate the interplay between on-farm practices to minimize raw product contamination, disinfection during processing, growth during distribution, and the role of microbiological testing in a holistic food safety management approach.

Example 3.7
Szabo et al. (2003) estimated the initial contamination level of *L. monocytogenes* on pre-cut lettuce, reductions using sanitized washing, and increases after packaging as well as during storage and distribution. For a given initial level of *L. monocytogenes* on lettuce and the expected level of growth (ΣI) during storage and distribution, the necessary reduction level to achieve a given FSO was determined.

For instance, given an initial population of $H_0 = 0.1$ $\log_{10}$ cfu/g and an estimated potential increase of $\Sigma I = 2.7$ $\log_{10}$ cfu/g during storage for 14 days at 8°C, a $\Sigma R \geq 0.8$ $\log_{10}$ cfu/g was deemed necessary to achieve the FSO value assumed to be set at 2 $\log_{10}$ cfu/g:

$$H_0 - \Sigma R + \Sigma I = 2 \rightarrow 0.1 - 0.8 + 2.7 = 2$$

Using this approach, the process can be considered to achieve the FSO exactly. However, in order to fully appreciate the impact of process variation it is necessary to move from point estimates to distributions that describe the variability of control measures in the risk management framework; for illustration purposes, the data from Szabo et al. (2003) are used in the example that follows.

Assume the standard deviation for ΣI is 0.59, and assume the log increase of *L. monocytogenes* is normally distributed. For ease of calculation and explanation, H_0 and ΣR levels do not include variation. Because of the distribution of ΣI, the producer must target a lower average level of *L. monocytogenes* in the finished product to reliably meet the FSO. If the same average level was targeted (i.e., FSO = 2 $\log_{10}$ cfu/g), 50% of the products would be above the FSO to some extent. The processor can consider other sanitizing wash methods to provide a greater reduction step to help to achieve the FSO through process control. The level of reduction needed to achieve different levels of conformity is presented in Table 3.4. For example, if the ΣR is 2.62, the proportion product above 2 logs, for a log normal distribution with mean log 0.18 and standard deviation 0.59 is 0.1%.

The next step in this example is to include variability in the process for all process stages. This section assumes variation for H_0, ΣI and ΣR (see values in Table 3.5). The resulting total describes the distribution of levels of *L. monocytogenes* in packages of fresh cut lettuce at the point of consumption, and is equal to the sum of the log means for H_0, ΣI and ΣR. The mean is not a correct indicator of the risk without considering the variance. The variance of the total distribution equals the sum of the variances, thus the standard deviation is the square root of the sum of the squares of the standard deviations. The distributions are illustrated in Fig. 3.9. Given this distribution of outcomes, the proportion of packages of lettuce not meeting an FSO = 2 log cfu/g in this example is 0.2%.

Table 3.4 Results of various levels of reduction (ΣR) on the proportion of defective units (P) with a standard deviation for the increase of 0.59, assuming the log increase is normally distributed

Reduction	Conceptual equation	Probability that FSO = 2 is exceeded
[ΣR]	[H_0−ΣR + ΣI]	[P (H_0−ΣR + ΣI) >2 (sd = 0.59)]
0.8	0.1–0.8 + 2.7 = 2	0.5 (50%)
1.2	0.1–1.2 + 2.7 = 1.6	0.25 (25%)
1.77	0.1–1.77 + 2.7 = 1.03	0.05 (5%)
2.17	0.1–2.17 + 2.7 = 0.63	0.01 (1%)
2.62	0.1–2.62 + 2.7 = 0.18	0.001 (0.1%)

Also see ICMSF (2011), and Zwietering et al. (2010)
Note: The proportion above the FSO is determined by the cumulative normal distribution $F(2;\mu,\sigma^2)$, which is calculated in Excel by 1-NORMDIST(2,x,s,1). For example, for the last line =1−NORMDIST(2,0.18,0.59,1) = 0.001019

Table 3.5 Results on the proportion of products that do not meet the Food Safety Objective (packages of fresh cut lettuce calculated to have greater than 2 log cfu/g *Listeria monocytogenes* present at the point of consumption), with various mean log and standard deviation values for H_0, ΣI and ΣR

	H_0	ΣR	ΣI		Total[a]	
Mean log	−2.5	1.4	2.7		−1.2	H_0−ΣR+ΣI
sd	0.80	0.50	0.59		1.11	sd = sqrt(sd_1^2 + sd_2^2 + sd_3^2)
				P(>FSO)	0.2%	

Also see ICMSF (2011), and Zwietering et al. (2010)
[a]The level (log cfu/g) of *L. monocytogenes* present in a package of lettuce at the point of consumption

3.5.2 Special Cases Requiring a Refined Approach to the Use of the Equation

One obvious simplification of reality in the above examples of deploying the ICMSF conceptual equation across a value chain step is that ΣI in principle signifies the overall increase in hazard level on the basis of H_0. i.e. of the cells of the hazard present in the [single] raw material, ingredient and/or product intermediate that enters that step. As such, it would disregard introduction of a particular hazard from a new source, i.e. from other materials/ingredients or the environment of the operation, in the course of a step. This could for instance happen because of mixing of materials or contamination occurring during a step.

Mixing with other materials/ingredients may bring in additional cells of a hazard or it may dilute the hazard level per unit mass when no cells are present in the added material. In any case, such mixing could be considered as an "intended" event that is part of the safe product & process design and covered in the food safety management system put into operation. However, unintentional contamination from other sources may also add cells of a hazard to a food material/batch, but control measures should be put in place to adequately mitigate such events.

Notably, a number of different sources of unintended contamination may be considered:

- the same raw material from which H_0 is taken. Subsequent contamination within a step could be referred to as "*recontamination*" or "*dependent contamination"*.
- food contact surfaces, equipment for processing/handling the food material concerned as well as niches/locations in the overall environment of the operation. Contamination from these sources may be referred to as "*cross-contamination*" or "*independent contamination*".

Mixing and contamination events described above are discrete events in time that affect the hazard level H_0 and bring in hazard cells not previously part of H_0. As such, they require a tailored approach to use of the conceptual equation but also warrant consideration of the specific time sequence of events.

Discrete events may have a marked impact on the total number of cells of a hazard in a batch and thus on its potential impact on public health. Therefore, a good quantitative understanding of the underlying dynamics of a hazard in the overall step will be critical to establishing a safe product and process design and for implementing this adequately in a food safety management system.

Notes:

- A discrete event such as partitioning does not cause a change in hazard level per unit mass that originates from H_0
- Discrete events such as cleaning or peeling may remove product mass and as such could reduce the hazard level/H_0

Mathematically, increases from H_0 (e.g. due to growth) as well as decreases from H_0 (such as reduction due to inactivation or dilution) can be represented as a continuum, but recontamination and cross-contamination will cause a discontinuous increase in hazard level. Zwietering (2005) therefore proposed to account separately for increases that are either due to growth (G) or to cross-/re-contamination, amending the conceptual equation to:

$$H_0 - \Sigma R + \Sigma G + \Sigma C \leq \text{FSO or PO} \tag{3.3}$$

Where:

ΣG = Total (cumulative) increase by (exponential) growth of the hazard
ΣC = Total (cumulative) increase of the hazard through cross-/re-contamination

It should be noted that the impact of a reduction event or of growth on the concentration of cells in a mass of food material is independent of the initial hazard level (Zwietering 2005). For instance, a 6D reduction by heating remains the same whether the hazard is present at 1000 cells/g or at 1 cell/g, namely a 6 $\log_{10}$ reduction in the initial concentration. Likewise, 100-fold growth over a step causes a 2 $\log_{10}$ increase in the initial concentration whether this starts at 1 cell/g or 1000 cells/g. Thus, growth and inactivation are "additive" on a logarithmic scale.

In contrast, discrete events such as cross-contamination/re-contamination (see Examples 3.8 and 3.9) as well as mixing (Example 3.10) are "additive" on a linear scale, not on a logarithmic scale.

Note that a direct way to estimate the log values for the various parameters in the conceptual equation, including those for increase through growth and contamination, may be based on the use of characteristic numbers as described by Zwietering (2005).

A contamination event in the course of a step will involve transfer of cells of a hazard onto a mass of food material that is being handled/processed in an operation. It will be important to understand the impact of the contamination on the overall concentration of the hazard in the food material (as this potentially impacts consumer risk) and the timing of the contamination event.

Should a contamination event occur before a significant reduction step, the impact of the contamination on the overall hazard level may be inconsequential or it may be not, as it depends on the magnitudes of reduction and contamination. The two examples below will illustrate these special cases for contamination pre and post an inactivation treatment.

Example 3.8

A batch of 1000 kg has an initial hazard concentration (H_0) of 10 cells/g (1 $\log_{10}$). The total batch of 1000 kg then contains 7 $\log_{10}$ cells. Assume that a contamination occurs with 1 g of material containing 1000 cells/g (3 $\log_{10}$ cells/g). This event will add 1000 cells to the batch, resulting in a level of 10,001,000 cells in 1000.001 kg. The new hazard level concentration is 10.001 cells/g (log 1.000043 $\log_{10}$). When this batch is subjected to a 6D reduction after the contamination event, this small increase does not represent a significant impact on public health, since the resulting hazard level is −5 $\log_{10}$ and (theoretically) −4.99996 pre- and post- contamination, respectively.

Example 3.9

However, should the batch be contaminated with the same amount of mass (1 g) and cells (1000 cells) after the 6D reduction step, so at the point where the incoming hazard level has been reduced from 1 $\log_{10}$ to −5 $\log_{10}$, the contamination will add 1000 cells (3 $\log_{10}$) to a batch with overall 10 cells of the hazard post-processing. Having 1010 cells/batch of 1000.001 kg equates to a concentration of 0.00101 cfu/g (equal to −2.99568 $\log_{10}$/g). Going from −5 $\log_{10}$/g to this new concentration close to −3 $\log_{10}$/g represents a ~2 log increase in overall concentration and it may represent a significant impact on public health depending on the severity of the hazard.

Interestingly, for the batch and contamination event provided in the last example above, there is a tipping point in terms of importance of the magnitude of post-process contamination relative to the magnitude of hazard reduction. This is illustrated in Fig. 3.10 for a 1000 kg batch of food contaminated at 1 $\log_{10}$/g, subject to an inactivation treatment varying between 0 and 8D and subsequent contamination with 1 g containing the hazard at 3 $\log_{10}$/g. This shows that for inactivation levels below 4D, the hazard level remaining from H_0 greatly determines the ultimate level of the hazard, whereas for inactivation levels over 4D, the post-process contamination determines the final hazard level.

Example 3.10

Mixing of raw materials and/or ingredients with an incoming material in a step may increase the concentration of a particular hazard in a food material/batch by adding the hazard from a new source. Quantifying the impact of this on the hazard level dynamics can be using Eq. 3, i.e. the conceptual equation of the ICMSF amended according to Zwietering (2005).

Assume that 100 g of a spice mixture is added to a batch of 100 kg of salad vegetables. The initial hazard level in the salad vegetables (H_0) is -2 $\log_{10}$/g, while the spices mixture contains 5 $\log_{10}$ cfu/g of a hazard (so 7 $\log_{10}$ cells in total). Pre-mixing, the hazard level is 1000 cells in the batch. Post-mixing, the level is 10,001,000 in the batch of 100.1 kg, or 99.91 cfu/g (1.999 $\log_{10}$/g). So the increase in hazard level is 3.999 $\log_{10}$ (from -2 to 1.999 $\log_{10}$/g). In case the spice mixture would be contaminated at 2 $\log_{10}$ cfu/g, the post-mixing level would be 0.11 cfu/g (-0.96 $\log_{10}$/g), i.e. an increase of 1.041 $\log_{10}$.

3.6 Process and Product Criteria

A performance criterion is met by implementing process criteria such as time and temperature of a heat treatment, and/or product criteria such as the water activity of the product, alone or in combination across the step in the value chain concerned, to achieve control over a specific hazard.

For example, the process criterion for milk pasteurization is 71.7 °C for 15 s and has been adopted as a regulatory requirement in numerous countries. It defines the processing conditions considered necessary to ensure inactivation of *Coxiella burnetti.* This process criterion will also ensure elimination of other non-sporeforming pathogenic enteric bacteria known to occur in raw milk.

An example of a product criterion is pH $\leq$ 4.6 in canned shelf-stable acidified foods as parameter known to inhibit growth of *C. botulinum.*

In the case of minimally processed refrigerated foods, combinations of process and product criteria have been proposed to ensure the safety of products with respect to non-proteolytic *C. botulinum*. In these cases heat-treatments milder than 90 °C/10 min in combination with other factors such as reduced pH, water activity or shelf-life (AMCSF 1992; Gould 1999) are recommended.

Other examples of linking product and process criteria to performance criteria are provided in the Principles and Guidelines for the Conduct of Microbiological Risk Management issued by Codex Alimentarius (CAC 2007a).

3.7 The Use of Microbiological Sampling and Performance Criteria

Increasingly, it is realized that food safety management systems based on preventing hazards through GHP and HACCP are much more effective in ensuring safe foods than end-product testing.

In the ICMSF scheme for managing microbiological risks (Chap. 1, Fig. 1.2) two uses of microbiological criteria are identified:

(a) To validate that control measures meet the performance criteria.
(b) To determine acceptability of a food when no more effective means of providing such assurance of safety are available, i.e. in the absence of knowledge that GHP and HACCP have been properly applied (see Chap. 4 for more details)

Different control measures or options can be applied to manufacture safe foods to meet the FSO or PO, as appropriate. The equivalence of these measures, in comparison to the established performance criterion, needs however to be established. For a number of processes and products this can be expressed in terms of frequency or concentration of a microbiological hazard in the food.

Traditionally the performance of sampling plans and microbiological criteria has been expressed in terms of "defect rate". The proportion of defective samples can be established by using the distribution of bacteria for relating the performance of attribute plans to the concentration of a hazard (Foster 1971; Legan et al. 2000). Homogeneous distribution or random sampling according to Chap. 7 has to be assumed.

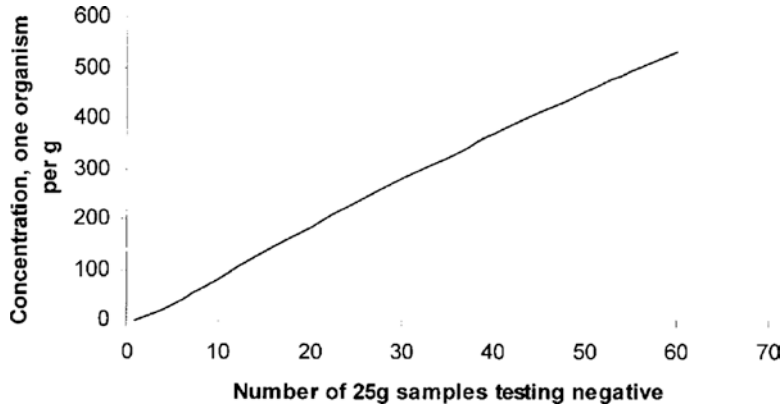


Fig. 3.11 Number of 25 g samples testing negative to have 95% confidence in meeting standard

Figure 3.11 shows the number of 25 g samples that may be needed and tested negative to be able to conclude (with a probability of 95%) that the concentration of organisms per grams of food was at a given level or below. For example:

$$13 \times 25\text{g negative corresponds to} < 1\text{cell} / 125\text{g}$$
$$29 \times 25\text{g negative corresponds to} < 1\text{cell} / 250\text{g}$$
$$60 \times 25\text{g negative corresponds to} < 1\text{cell} / 500\text{g}$$

Using this approach, microbiological testing can be used to ensure that the concentration of the hazard in an ingredient (H_0) does not exceed a given concentration (assuming a homogeneous distribution or random sampling as per Chap. 7). In this way a microbiological criterion can be used as a control measure to help meet a performance criterion and, thereby, an FSO as in Example 3.5.

It must, however, be realized that for a number of foods the application of effective control measures will allow the achievement of much lower defect rates and that under such circumstances testing remains questionable.

3.8 Quantitative Performance of Attributes Sampling Plans

The attribute assessed in attributes sampling plans in food microbiology is frequently based on the presence or absence of the microorganism of concern in a defined quantity of the sample, or series of samples, of the product (e.g., not detected or "negative" in five samples of 25 g each). However, the attribute is sometimes based on whether the concentration of microorganisms in the sample is above or below a limit (e.g., $<10^2$ cfu/g).

It is useful to understand how probable it is that a given sampling plan will detect a certain level of contamination in the product and thus reject a non-conforming batch. This is known as the *performance* of the sampling plan. It has been demonstrated that contamination is often not homogenously distributed within a lot. In other words, single distribution does not characterize the population, but rather a mixture of multiple distributions. At the scale of a lot or between lots, the mean concentration is usually not constant but varies according to a lognormal distribution. However, at the local scale of a sample, the mean concentration can be considered constant, in which case the number of colony forming units in a sample varies randomly according to the Poisson distribution.

Frequently, most samples of a contaminated lot will test negative, with only a few testing positive. These few, however, may be capable of causing illness. Therefore, when selecting or designing an attributes sampling plan, the intent is to ensure that the *average* concentration in the batch is sufficiently low so that, within a specified level of confidence that accounts for variation, no samples from the batch contain unacceptable levels.

When an attributes sampling plan is based on a detection of a microorganism in a defined quantity of food, the absence of any positive result is often misinterpreted as demonstrating the total absence of contaminants in the whole lot. A more appropriate interpretation is that presence/absence testing based on enrichment methods involves the same concept as a "most probable number" method, in which replicates of a single dilution of the sample are tested. Thus, absence of a positive result suggests only that the contamination level is below that which the sampling plan is able to reliably detect. The performance or likelihood of a sampling plan to detect a microorganism can be determined (Legan et al. 2000; van Schothorst et al. 2009). The method described by van Schothorst et al. (2009) is more appropriate for sampling plans involving enrichment and is outlined below.

It may be tempting to infer that a negative result for a sample can be used to calculate the concentration on the basis of simple probability; e.g., absence in 25 g suggests that the concentration is <1 cell/25 g or <0.04 cells/g, and absence in five samples of 25 g infers that the concentration is <0.008 cells/g. This simplistic approach assumes that cells are homogenously distributed in the lot, and even in these situations of low to very low pathogen concentration, the probability of detecting a positive in the sample is not 100% but rather only 63%. Variation in the concentration of cells in the lot and random aspects of sampling small particles (cells) in large samples must be considered. However, taking more random samples provides more confidence that the results are representative of the entire lot, but cannot guarantee detection.

At the very low pathogen concentration levels typically considered in presence/absence testing, assuming a continuous distribution like the lognormal is inappropriate because organisms are discrete. Discrete distributions like the Poisson are more appropriate because a sample either has no organisms or a countable number of organisms. Even if the cells are evenly distributed throughout the lot, the result is affected by chance events relating to the position of the cell relative to where the material is sampled. Thus, even when the true concentration in a sample is below the acceptable limit, a sample unit could contain a cell and the batch be rejected with a c = 0 sampling plan.

Similarly, a series of samples may fail to include a cell even if simple probability would suggest that at the concentration present, a cell would be expected to be detected among the total volume of sample analyzed. This effect is less pronounced when a higher concentration of cells is acceptable, e.g., when the attribute is set at <100 cells/g, as opposed to absent in the sample. This is because the sampling error is larger when fewer items are observed in the sample. In Poisson processes, the standard deviation is equal to the square root of the mean number of target cells/sample. Presence/absence methods are based on the observation of one, or at most, a few cells. Thus, whereas the standard deviation associated with a count of 100 cells is ±10%, for a test involving observation of single cell the standard deviation approaches 100%.

It has been demonstrated that the concentration of microorganisms frequently follows a log-normal distribution in foods (Jarvis 2008). Therefore the normal distribution of log counts can be used to estimate the proportion of defective samples in a lot if the overall geometric mean (the term "mean" refers to geometric mean throughout the rest of this chapter) and standard deviation are known or can be inferred. In reality, the standard deviation can never be truly known. It must be estimated. However, estimates of these values can be used to determine the relative probability of accepting a defective lot of food for a given sampling plan.

A sampling plan can never assess the mean concentration of the entire lot with complete accuracy. It can only estimate the concentration at a selected level of confidence. To assess the performance of a sampling plan, one needs to know the number and size of samples tested, and assume the variability

in concentration of cells within the lot. The Poisson effect in sampling can also be accounted for in interpreting the detection threshold of a specified attributes sampling plan. Information on sampling plans as well as a spreadsheet tool enabling the necessary calculations and including consideration of the Poisson effect are available (ICMSF 2015b).

ICMSF (2011) used the tool identify the geometric mean that results in a 5% probability of lot acceptance under different sampling plans recommended in this Book 8 using a range of standard deviations. While the true standard deviation of the distribution of concentration of contaminants in a lot is unknown, thus the tables included in Appendix A of ICMSF (2011) consider a range of distributions of cell concentration for illustration purposes. For example, the standard deviation of the distribution of cell concentrations in a well-mixed product such as milk may be lower than that for a product in which ingredient quality or process hygiene could vary over the production run. The standard deviations used apply to distribution of cell concentrations and do not include variation associated with analytical methods.

3.9 Default Values

In the absence of an FSO it may be appropriate to establish default criteria for certain control measures. These fail-safe criteria, developed by advisory bodies of experts or control authorities, are intended to control hazards under "worst-case" situations and will, of necessity, be less flexible. They may assume a higher than normal initial level of a hazard (H_0) and/or an increase (Σ I) during processing or prior to consumption.

An example of default criteria is the cooking of roast beef to an internal temperature of 62.8 °C to destroy enteric pathogens such as salmonellae and pathogenic *E. coli* (USDA 1999, Appendix A). Another example is the in-pack thermal treatment for 10 min at an internal temperature of 90 °C of ready-to-eat chilled foods with extended shelf-life to destroy non-proteolytic *Clostridium botulinum* (ACMSF 1992; Gould 1999).

Default values provide a safe harbor for food operators lacking either the resources or the desire to develop information necessary for alternative criteria that may be more appropriate for their specific operation or product.

3.10 Process Validation

Control of food operations depends upon operator knowledge and the conditions that influence the production of safe and unsafe food. A considerable amount of information is available in the literature and other sources. For new, novel processes it may be necessary to develop information to verify the efficacy of the control measures. Some operations may be so unique and different from other operations producing similar foods that control is less certain. In other situations an operator may wish to use minimal processing techniques for improved product quality or reduced cost. In such instances it may be necessary to validate the efficacy of the adopted control measures.

Validation can involve:

1. Developing data through challenge tests in the laboratory that are intended to mimic the conditions of operation;
2. Collecting data during normal processing in the food operation;
3. Comparison with similar processes/products;
4. Other expert knowledge.

Each method has its strengths and weaknesses and in certain cases more than one method is best used for validation. Data developed through laboratory challenge tests can involve the food, culture media, or other material that may be appropriate. Challenge studies in a food processing environment can provide a higher degree of assurance concerning the ability to meet performance criteria; however, this requires the use of surrogate test microorganisms (see below). Pathogenic microorganisms should never be introduced into the food production or processing environment for the purpose of process validation. In some cases, it may be possible to follow changes in the population of naturally occurring pathogens throughout a process. Such studies, for example, could be conducted during the preparation and processing of raw agricultural commodities into ready-to-eat foods. Ideally, validation could involve laboratory challenge tests with pathogens in the laboratory and then re-validation after the control measures have been implemented. This, however, may be impractical in situations where the prevalence of a pathogen is very low and large numbers of samples are necessary to develop meaningful data.

3.10.1 Laboratory Challenge Tests

When conducting laboratory challenge studies, factors such as the following should be considered:

- **Intrinsic resistance of the pathogen.** Studies to evaluate the resistance of a pathogen to different parameters (e.g., heat, cold, acid) that may be incorporated into a control measure should be performed using several strains (e.g., five or more) including outbreak-associated isolates from the food in question. Resistance of the strains used for testing is a key factor when establishing effective control parameters. The inocula should be prepared under conditions that yield resistance of the pathogen appropriate to the process. For example, vegetative cells of salmonellae and pathogenic *E. coli* should be used that demonstrate a maximum resistance to heat and acidic conditions when in the stationary phase after having been grown at elevated temperatures. Sufficient numbers of the pathogen (e.g., cells, spores, viral particles, oocysts) should be used to eliminate biovariability effects.
- Strains to be tested should not include isolates with unrealistically extreme resistances or growth characteristics when these are not associated with public health concerns for the particular food or situation at hand. For example, *Salmonella* Senftenberg 775W is appropriate to evaluate survival of *Salmonella* spp. during the bean roasting step of chocolate production, since heat-resistant *Salmonella* spp. have been associated with outbreaks involving chocolate and roasting is the single most important pathogen inactivation step for this situation (ICMSF 2005). Thus, the test organism represents a particularly heat resistant contaminant to validate the appropriateness of the thermal process design with. However, *Salmonella* spp. associated to outbreaks involving liquid egg production are not particularly heat-resistant (ICMSF 2005) and using *Salmonella* Senftenberg 775W for design validation is not appropriate.
- **Use of non-pathogens for validation.** Validation of control measures in a food operation can be accomplished through the use of non-pathogenic microorganisms if they have been shown to have the same growth pattern or resistance as the pathogen of concern. For example, *Enterococcus faecium* (strain NRRL B-2354) is recommended as a surrogate for *S.* Enteritidis PT30 in thermal process validations for almonds (Jeong et al. 2011), dairy products, meat (Annous and Kozempel 1998) and juice Piyasena et al. 2003).
- **Composition of the food.** Composition of the food can affect inactivation, survival and/or growth of pathogens and therefore must be known and taken into account. Factors such as pH, a_w, Eh, humectants, acidulants, solutes, antimicrobials, substrates, competing microflora can affect the chemical and physical properties of the food and subsequently the pathogen of concern. Normal

variation in the concentration and distribution of food constituents and microorganisms must also be known and understood.

– **Conditions of storage, distribution, preparation for use.** Factors affecting the safety of a food during storage, distribution and preparation for use must be identified and controlled. Information on the intended use and an estimate of likely misuse of the product may be necessary. Examples of parameters that often have a significant effect include time and temperature, the potential for contamination, and faulty preparation before consumption.

3.10.2 Data Collected From Food Operations

A considerable amount of data can be collected from a food operation to better understand the potential microbial hazards. The data can consist of a variety of chemical, physical and microbiological measurements. For example, if the chemical composition of a food is known as it undergoes processing, estimates can be made of the potential for certain pathogens to survive or multiply. Similarly, measurements of processing times and temperatures must be understood if the potential for survival and growth during processing is to be estimated. While generalizations often can be made from published data, the source and type of raw materials may differ among food operators. The best means to establish H_0 is to analyze raw material samples from an operation, over a period of time to take account of potential seasonal variability. Perhaps one of the most comprehensive analyses of low concentrations of microorganisms in food ingredients comes from Barker et al. (2002). This work used a Bayesian framework to model belief concerning the concentration of spores of non-proteolytic *Clostridium botulinum* in materials used during the manufacture of minimal processed chilled meals in the UK. Posterior belief about the spore load centered on a range of concentration of 1–10 spores/kg and the beliefs about the spore loads can be used for numerical analysis and risk assessments.

Opportunities also may exist for collecting microbial data from samples collected as a food is being processed. Such in-plant data can be used to validate a process or to verify results obtained in the laboratory. Measuring changes in the population of a pathogen in raw materials as the food is being processed provides an ideal situation for in-plant validation. For a variety of reasons, however, it may be necessary to measure changes in the population of a non-pathogen that has similar or greater resistance to the pathogen. This may be necessary, for example, when the numbers or prevalence of the pathogen are too low to develop meaningful data. The variability in a pathogen population can be influenced, for example, by season, geographic location of the operation, source and type of raw materials, and processing conditions. These and other factors should be considered when collecting data for use in process validation.

3.10.3 Process Variability

The variability that occurs in a food operation must be considered when establishing the critical limits associated with control measures. Examples of factors that can influence variability of a process include equipment performance and reliability, integrity of container seals, processing times and temperatures, pH, humidity, flow rates and turbulence.

It is essential that the variability of process parameters and product formulation be taken in account when setting critical limits. In general terms, the critical limits at a CCP for a process CCP operating under a high degree of control (low variability) can be closer to the conditions necessary for control of a hazard as discussed above. Conversely, the critical limits for a less controlled process (high variability) must be more conservative and more restrictive. In other words, critical limits must be based

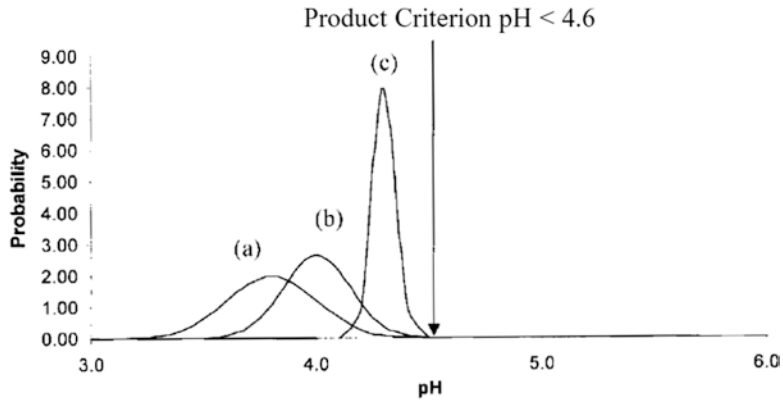


Fig. 3.12 Set point depends on the variability in the process

on the capability of the process to achieve a given criterion under normal operating conditions taking into account variability. Monitoring and verification procedures specified in a HACCP plan should be designed to determine when the process is operating outside this normal variability and so appropriate corrective actions can be taken.

These principles are illustrated in Fig. 3.12. Three different process/product capabilities are illustrated, each of which must meet a product criterion of < pH 4.6 to ensure the safety of a high acid product with respect to *Clostridium botulinum.* In the first example, there is poor control of final product pH and a high variation (distribution a.) hence the operating target pH (mean) or 'set point' must be at pH 3.8 to be sure that <pH 4.6 will always be met. In the second example, there is better control of the process and resulting final product pH (distribution b.); hence, the 'set point' for the process is pH 4.0 and closer to the required product criterion. In the final example there is excellent control of the process (distribution c.) and the 'set point' can be at pH 4.3.

An effective process control system is a key element in the management of food safety and can, in addition, provide economic benefits. Processes under control are less likely to yield foods that will cause harm to consumers. Food processors who understand the factors that can cause variability in their operation will have established monitoring systems to detect and prevent unacceptable loss through inefficiencies, reduced yield, or poor quality. Similarly, by incorporating the elements of GHP and HACCP into their process control systems food operators can ensure the production of safe foods. Whether for economic gain or food safety, criteria are established at selected points in the operation to enable the operator to assess control. The operation is considered under control while established criteria are being met. If not, adjustments must be made to bring the process back under control. A number of statistical tools that can be used to aid in the evaluation of process control and trend analysis both for microbiological testing and for the physical and chemical parameters (see Chap. 13). Through knowledge of the process and use of the data, operators can plan for and achieve continuous improvement thereby further reducing variability and achieving greater control.

Process control systems can involve two types of measurement, real time and delayed time. In the former, data are collected and used to adjust processes during the operation. Examples include measurements for pH, temperature and humidity. Ideally, there is continuous feedback to provide automatic adjustment as the operation proceeds. Delayed time measurements do not yield data that permit adjustment to an ongoing operation. Examples include measurements using conventional microbiological methods and certain chemical analyses. Due to the time elapsing between when samples are collected and results are obtained these methodologies yield historical data and document what has happened rather than what is happening. While of less value for current production the data can be used to detect trends and with proper adjustments reduce the likelihood that future lots will be unacceptable. These concepts will be discussed more fully in Chap. 13.

3.11 Monitoring and Verifying Control Measures

After effective control measures have been established, it is necessary to establish procedures to monitor each CCP in HACCP plans and verify that the control measures are being implemented as planned. Monitoring and verification can consist of a variety of measurements, such as:

- Sensory assessments based on visual, aroma, taste, touch and sound;
- Chemical measurements, such as for sodium chloride, acetic acid, or water content;
- Physical measurements, such as pH, a_w, humidity, temperature;
- Time measurements;
- Packaging, such as integrity of the container closure (e.g., hermetical seal);
- Records for incoming raw materials (e.g., record showing an ingredient is from an approved source or region);
- Microbial tests, including tests for toxic metabolites;
- Environmental sampling (see Chap. 12).

It is important to note that the microbiological safety of most foods can be assessed by a variety of methods other than by microbiological testing. These determinations can be used for monitoring or verifying that the specified control measures in a food operation are /or have been implemented correctly. For example, measurements of pH and/or acid content are commonly used for monitoring fermentation processes and foods that are acidified.

Some of these methods also are very satisfactory for assessing the acceptability of foods at port of entry or upon receipt for use in a food operation. For example, the acceptability of a high acid canned shelf-stable food can be easily determined by performing a pH measurement. Many foods of relatively low moisture that are intended to be distributed and sold at ambient temperature can be assessed by a measurement of a_w or moisture content.(see Chap. 4). Food operators become very familiar with the normal appearance, aroma, and feel of the foods with which they are involved. Foods that appear atypical should raise doubts in the operator's mind and indicate a need for additional evaluation.

3.12 Examples of Control Options

Two examples will be used to demonstrate the relationship between performance criteria, product/process criteria, and microbiological criteria (Baird-Parker and Tompkin 2000). The first, is a recommendation to industry by the UK Advisory Committee on the Microbiological Safety of Foods (ACMSF 1992) of four control options to control the risk of psychrotrophic *C. botulinum* in refrigerated cooked foods having an extended shelf life of more than 10 days. The recommendations were:

1. A heat treatment of 90 °C for 10 min, or equivalent lethality;
2. A pH of 5 or less throughout the food and throughout all components of complex foods;
3. An a_w of 0.97 or less throughout the food and throughout all components of complex foods
4. Combination of heat and preservative factors, which can be shown consistently to prevent growth and toxin production by non-proteolytic *C. botulinum*;

Option one is directed toward killing vegetative cells and spores of psychrotrophic strains of *C. botulinum* that may be present in the raw materials used in the production of the foodstuff. Options two and three are intended to prevent the growth of the organism and hence prevent toxin production. Option four could involve thermal destruction/thermal injury of spores and/or inhibitory factors to prevent the outgrowth of surviving *C. botulinum* spores.

Underlying each control option, is an unstated performance criterion. Thus, the performance criterion for option one could be stated as a 6D reduction of spores of psychrotrophic strains of

C. botulinum as this is the intended result of a heat treatment of 10 min at 90 °C. For options two and three the performance criterion could be stated as less than a 1 log increase of *C. botulinum* within the use-by-date when stored at the recommended storage temperature. The ACMSF report provides considerable background information on the likely occurrence of the hazard and factors that can be used for control.

The second example concerns the risk of *E. coli* O157:H7, and similar enteric foodborne pathogens in fermented sausages. In December 1994, an outbreak of foodborne illness caused by *E. coli* O157:H7 in a fermented sausage product occurred on the West coast of the U.S. In response, the U.S. Department of Agriculture established a requirement that all manufacturers use processes that control the risk of illness from *E. coli* O157:H7. In this case, the agency proposed a performance criterion (i.e., 5D kill of *E. coli* O157:H7) and left it to industry to decide how to satisfy the criterion and still produce products of acceptable quality. The agency's proposal of a 5D kill was based on very limited evidence suggesting that up to 1000/g of *E. coli* O157:H7 could occur in the raw meat used for processing. Industry sponsored research led to five options that were accepted by the agency (Nickelson et al. 1996). The five options were:

1. Apply an existing, approved heat treatment as specified in former USDA Regulation 9CFR 318.17 (i.e., heating to an internal temperature of 62.8 °C for 4 min or to a lower temperature for such time to obtain an equivalent level of safety).
2. Apply a process that is validated by research to cause a 5D kill of *E. coli* O157:H7 before the product is released for distribution.
3. Combine raw material testing with a process that is validated by research to cause a 2D kill of *E. coli* O157:H7 before the product is released for shipment. The sample procedure must ensure the level of *E. coli* O157:H7 in the raw sausage blend does not exceed 1/g. One such sampling procedure could consist of analysing 15 samples (25 g each) collected at the time of stuffing the meat blend into casings.
4. Apply a hold and test program for the finished product before distribution. Products intended to be heated before serving (e.g. pepperoni for pizza) would be sampled at a rate of 15 samples per lot. Products normally consumed without heating before serving (e.g., salami) would be sampled at a rate of 30 samples per lot. The analytical unit for each sample tested would consist of 25 g.
5. To allow for new technology or ideas, this option permits the use of alternate processes that provide the equivalent of a 5D reduction.

All of the options are intended to ensure the level of *E. coli* O157:H7 is 1 cell/100 g or less when the products are released for distribution. This was considered by the agency at the time to provide an acceptable level of consumer protection for this class of product. The five options include process criteria, performance criteria and microbiological criteria. Options 1 and 2 assume an initial level of 1000 *E. coli* O157:H7/g in the raw sausage blend. The process criterion for option one (heating to an internal temperature of 62.8 °C and holding for 4 min) is derived from an existing regulation for roast beef and is based on research data demonstrating a 5D kill of salmonellae and *E. coli* O157:H7 in beef. The 4 min hold time was an added requirement since the roast beef regulation does not require a hold time at 62.8 °C. Processors choosing this option would not take advantage of the faster rate of kill that would occur with the reduced pH of a fermented product. The performance criterion in option two, specifies a 5D kill of *E. coli* O157:H7. To satisfy this option, the processing plant must have on file, and available for review, research data which validates that the process being used will achieve a 5D kill. The validation research must have been developed with a protocol approved by the USDA.

Option three incorporates both a reduction step and an elimination step based on microbiological criteria. It involves a performance criterion of a 2D kill, in combination with microbiological testing to verify that the level of *E. coli* O157:H7, in each production lot, does not exceed 1/g in the raw sausage blend; collectively, the net result is equivalent to the 5D kill of option 2 which assumed an initial

level of 1000/g in the raw sausage blend. Subsequent industry sponsored research has demonstrated that fifteen 25 g samples of raw sausage blend can be composited for analysis with no significant loss in sensitivity of detection. If, for example, a processor elects to collect 15 samples from across a lot during stuffing and have each tested using a 25 g analytical unit (375 g total), then this would provide 95% probability that the level *of E. coli* O157:H7 in the blend is no more than 1 cell/125 g if a negative result is obtained (Foster 1971). While this may be a prudent sampling plan for some operations, this level of sampling exceeds by approximately 100-fold the detection level required in option 3.

Option four establishes microbiological criteria for finished product and assumes no prior knowledge of the level of *E. coli* O157:H7 in the raw sausage blend or the lethality of the process. Reliance is placed solely on the use of a sampling plan that may detect *E. coli* O157:H7, if it is present in the finished product. The sampling plans are based upon ICMSF Book 2 (ICMSF 1986) and an assignment of *E. coli* O157:H7 to cases 13 and 14 for pepperoni and salami, respectively. Case 13 involves $n = 15$ and $c = 0$. Case 14 involves $n = 30$ and $c = 0$. A negative result with fifteen 25 g samples (375 g total) provides a 95% probability of no more than 1 cell/125 g. A negative result with thirty 25 g samples (750 g total) provides a 95% probability of no more than 1 cell/250 g (Foster 1971).

3.13 Assessing Equivalency of Food Safety Management Systems

Food safety management systems based upon an FSO and the use of performance criteria provide greater flexibility in how food operators can control hazards. An assessment for equivalency of systems that use performance criteria may require a holistic approach when the food operation involves multiple steps. In another situation an assessment for equivalency may be limited to a single step in a process (e. g., pasteurization). The assessment should involve a review of data that demonstrate the scientific basis for the alternative process and processing records that demonstrate the control measures are being implemented as planned. Finally, the product must be able to meet any acceptance criteria that may have been established for the product.

It should be evident that the ALOP (or TLR), FSO, PO and risk-based measures subsequently established by a country can serve as one basis for assessing the equivalency of a country's food control system.

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Chapter 4
Selection and Use of Acceptance Criteria

4.1 Introduction

The global trade in food is extensive as demonstrated by statistics on food trade. For example, the value of world trade flow between countries in agricultural produce alone for 2008 ranged between 1060 billion and 1105 billion US dollars (FAO 2011). In 2014, the USA imported approximately 61 million metric tons of food products across 13 categories (excluding live animal imports) (ERS 2015). In addition to these country-to-country food product flows, food is traded within each country between food businesses in large volumes that are difficult to estimate. All these food products should be safe and of suitable quality. However, the safety of a product is not verifiable by sight and smell, and therefore food businesses and consumers must rely on their supplier to deliver raw materials of acceptable safety and quality or else rely on the effectiveness of government food controls.

In many instances, a food business or a government has no reliable means to check the safety of all incoming foods. In recognition of this limitation, commercial partners usually enter into a "business deal"where product liability is an important issue. Consequently, commercial buyers can impose specifications for the food being purchased along with a variety of options to verify supplier compliance. For similar reasons, trade in food between countries is usually subject to export and import controls developed by mutual agreement between trading governments that can lead to different forms of verification. However, in the case of governments, controls should be based on international standards, e.g. Codex Alimentarius, or otherwise be based on science and applied only to the extent necessary to protect human, animal or plant life or health (WTO 1995).

This chapter will discuss the use of criteria to determine the acceptability of individual lots or consignments of food and also the acceptability of the food processes producing them. Criteria for assessing the acceptability of a lot of food can take several forms and be based on sensory, chemical, physical, or microbiological parameters. Lot acceptance criteria should include additional information, for example, the number of samples to be collected, how and where the samples are collected and held prior to analysis, the analytical unit, the method of analysis, and what is considered acceptable. The acceptability of a food operation can be evaluated through inspections or audits by commercial buyers and control authorities. The results of an inspection or audit can also be used as a basis for decisions on whether or not a food should be sampled.

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4.2 Equivalence

For foods received at a port-of-entry, the origin (i.e., country or region) of the food is an important factor influencing acceptance. It is impossible for the control authorities of an importing country to inspect and approve foods while they are being produced in another country or region. Therefore, reliance must be placed on the sanitary measures associated with the food inspection and certification system in the exporting country or region. Thus, to facilitate the movement of foods across international borders, it is necessary to develop a mechanism for acceptance based on mutual agreement between the authorities in the participating countries. The equivalency concept was introduced in the SPS Agreement (WTO 1995). It recognizes that it is not always possible for importing and exporting countries to have identical inspection and certification systems but nevertheless, it is necessary to assess whether they result in an equivalent level of consumer protection. Equivalence, as defined by Codex, is "the state wherein sanitary measures applied in an exporting country, though different from the measures applied in an importing country achieve, as demonstrated by the exporting country, the importing country's appropriate level of sanitary protection" (ALOP) (Codex Alimentarius 2003).

The burden of demonstrating equivalence is on the exporting country. However, the importing country's sanitary measure must also meet its own ALOP and it must also be able to demonstrate this. If equivalency can be agreed, then further application of acceptance criteria at the port of entry may not be necessary. The procedure for the determination of equivalence established by Codex Alimentarius involves obligations for both importing and exporting countries. For further detail the reader is directed to the relevant Codex standard (Codex Alimentarius 2003).

One of the major hurdles in the establishment of equivalence of different sanitary systems is the requirement to demonstrate that a sanitary measure or set of measures meets the ALOP of the importing country. The SPS agreement allows for ALOPs that are stated either qualitatively or quantitatively, however, few countries have articulated an ALOP and even when ALOPs have been articulated, they are rarely quantitative. For example, Australia defines its ALOP as 'providing a high level of sanitary and phytosanitary protection, aimed at reducing risk to a very low level, but not to zero' (Commonwealth of Australia 2008). It is difficult for an exporting country to demonstrate equivalence against such a qualitative ALOP when a quantitative expression of what is meant by the phrase '...very low level, but not to zero' is not stipulated. Such decisions inevitably require qualitative judgements and negotiation between the importing and exporting country and this can lead to difficulties.

Codex Alimentarius has established the concept of 'objective basis of comparison' to further facilitate equivalence discussions. This allows the exporting country to demonstrate that its control measure(s) has the same expected outcome as the control measure(s) used in the importing country. This is based on the assumption that an importing country establishes its control measures to meet its ALOP, whether stated or not.

Chapters 1 and 2 discuss the interrelationship of ALOP and FSO. They describe the quantitative nature of the FSO and the fact that it provides or contributes to the ALOP. Similarly, a PO is also a quantitative expression of acceptable levels of microorganisms in a food and is designed to meet or contribute to the FSO. Consequently, if an importing country has articulated a FSO or, in certain circumstances a PO, this value can be used for the 'objective basis of comparison.' It is conceptually feasible for the exporting country to demonstrate equivalence of a sanitary measure or set of measures for a food based on achievement of the importing country's FSO or PO given their intrinsic link to the importing country's ALOP. Although the role of FSO and PO in the establishment of equivalence is not discussed in the relevant Codex standard (Codex Alimentarius 2003), it is a logical progression stemming from the adoption of the Codex standard on microbiological risk management (Codex Alimentarius 2007) and consistent with the stated uses of FSOs articulated in Annex 2 of that document.

4.3 Establishment of Acceptance Criteria

4.3.1 Types of Acceptance Criteria

Acceptance criteria can involve a variety of parameters (sensory, physical, chemical, microbiological) and generally fall into three categories:

Standard – a mandatory criterion that is part of a law or ordinance
Guideline – an advisory criterion issued by a control authority, industry association or a food producer to indicate what might be expected when best practices are applied
Specification – Part of a purchasing agreement between a buyer and supplier of a food; such criteria may be mandatory or advisory according to use

Governments are responsible for establishing food regulations and policies that ensure the safety of the foods for which they have regulatory responsibility. Thus, food control authorities serve as risk managers and can establish FSOs and, where appropriate, POs for hazards in foods. As described in Chap. 2, a FSO or a PO specifies the outcome expected of food processes that are under effective control measures. The outcome may be expressed as a frequency or maximum concentration of a microbiological hazard in the food. Furthermore, the FSO/PO is based on what is considered acceptable for consumer protection. Thus, a FSO/PO is a goal that food operators can use when designing and implementing their food safety management system, and thus can be used as the basis for establishing acceptance criteria.

4.3.1.1 Standards

Standards may be established for a wide variety of reasons but are best applied when risk to consumers is sufficiently high and compliance with the standard is essential for consumer protection. Standards are established by governments and define the parameters that processes or foods must meet to be in compliance with regulatory policies or regulations. Standards may take the form of performance objectives (PO), performance criteria (PC), and microbiological criteria (MC) (see Chap. 2). Process criteria (e.g., time and temperature) and sometimes product criteria (e.g., pH, water activity, etc. may also be described. These criteria are applied to both domestic and imported food either during lot acceptance or during process acceptance (domestic food production) and equivalence determination (imported food control systems). Standards that define microbiological criteria of importance to the safety or quality of a food should be established following the principles outlined by Codex (Codex Alimentarius 1997, 2013) and as further elaborated in Chap. 5 of this book. Thus, ideally microbiological standards should be developed following a quantitative risk assessment and establishment of a FSO or suitable PO. Some examples of such standards can be found in European Regulation 2073/2005 (as amended) on the Microbiological Criteria for Foods and the Australia New Zealand Food Standards Code – Standard 1.6.1 – Microbiological Limits for Food-F2011C00582.

4.3.1.2 Guidelines

Governments or other bodies can also specify risk management metrics in the form of guidelines and/or policies, often including additional details of what is considered best practice for a food production process. Guidelines can also be used by both industry and government as the basis for assessing the acceptability of a food operation or food lot.

Guidelines may be established for a process or product. In the case of a process (e.g., production of cheese, fermented sausage), the conditions that should be controlled are described so the resulting

food will be safe. A guideline for a product normally provides the product characteristics (e.g., pH, a_w) and microbiological criteria that the operator should strive for in order to produce an acceptable product. Such criteria describe the characteristics of a food when best practices are applied. Guidelines may be the preferred means to inform food operators that change is needed within a segment of the industry. In other situations, guidelines may be used to describe the conditions believed to correct a newly recognized microbiological problem where insufficient data prevent the establishment of a standard. Thus, guidelines can serve as an interim measure providing guidance to food operators until sufficient information and technology is available to establish standards. A guideline, alone, may be adequate to bring about the changes necessary to improve food safety and quality, thereby negating the need to establish standards. Examples of acceptance criteria in a guidance document on control of *Listeria monocytogenes* in ready-to-eat food (Health Canada 2011) and the guidelines for assessing the safety of ready-to-eat foods (HPA 2010). The first example was established by a regulatory government agency and the last example was developed by a non-regulatory government agency.

4.3.1.3 Specifications

Industry must produce foods that meet regulatory and customer requirements. To ensure compliance with these requirements and the business' own requirements for quality and safety, food businesses frequently establish purchase specifications for food ingredients and other materials. Implicit in the transfer process between buyer and supplier is the necessity for the buyer to make a decision whether to accept or reject ingredients, food and other materials. This decision involves a variety of factors, most important of which is the buyer's experience with the material being purchased. As buyers become more sophisticated and familiar with the risk(s) associated with certain foods they recognize the utility of having pre-established acceptance criteria that are agreed upon with their supplier(s). The purpose of such purchase specifications is to reduce the likelihood of accepting an unacceptable ingredient or food.

Purchase specifications define the expected characteristics of an ingredient so that when it is added, the final product will meet all requirements for safety, quality, wholesomeness and other requirements of concern to the buyer (e.g., cost, nutrition, compatibility with ingredient listing on label). It is now common for buyers along the food chain to establish their own specifications for the materials they purchase, particularly when the raw material or ingredient's safety is a CCP in the buyer's HACCP plan. Under certain circumstances, a food business may also specify the conditions of operation in the form of an operating instruction or best practices guideline. Examples of supplier specifications are not generally available publically; however, similar types of specifications have been set for the World Food Programme (WFP 2009).

4.4 Application of Acceptance Criteria

Control authorities are responsible for verifying compliance with standards. This can involve inspections of food businesses and, if deemed necessary, sampling and testing the food. Depending on the jurisdiction, certain food production operations that are in compliance with standards and other legislative requirements are typically granted a form of license that is required for continued operation. In other food businesses where licenses are not required, compliance may result in other rewards like a reduced inspection frequency. Failure to comply with standards and other regulatory requirements may result in sanctions such as the revocation or temporary suspension of a license, an official order to improve one or more practices or the recall of a food lot. In extreme circumstances where food safety is compromised to the point that public health is endangered, some jurisdictions allow control

authorities to close a food business pending compliance. Ideally, governments organize the results of their assessments to determine if trends indicate a need for change in the regulations or enforcement actions. These data can influence the control authority's stance on sampling and testing certain categories of food for compliance with established criteria.

Control authorities may also choose to verify compliance with guidelines. These are usually guidelines established by governments. Since guidelines are not mandatory, failure to comply may only result in recommendations for improvement. However, control authorities often collate the results of testing against guidelines as a way of judging general food chain compliance and whether a guideline should be converted into a standard following a risk assessment and the development of any necessary risk management metrics; e.g., FSO or PO. Food business's internal or external assessment against guidelines is a means of verifying compliance with accepted best practice. Compliance may also be necessary if the guideline is part of an industry certification scheme resulting in some form of certificate of compliance. Food businesses may also use acceptance criteria in guidelines to develop their specifications for suppliers.

Assurance that suppliers control the hazards is best accomplished through a system of auditing and approving a supplier's total system for the management of food safety and quality, such as described in Chap. 3. Food businesses that set specifications may choose to audit suppliers against those specifications either directly or indirectly via a third party certification body. This is more common when a new supplier is being evaluated and thereafter on a less frequent basis depending on the history of compliance of that supplier. Suppliers that are considered unacceptable are removed from the list of approved suppliers. Often suppliers of sensitive raw materials with regard to microbiological safety or spoilage, may have to test their products themselves against the buyer's specification and provide the buyer with a certificate of analysis for relevant parameters with each shipment to verify compliance. The buyer may also analyze some raw materials after they are received. Tests that are typically performed may involve a variety of parameters, such as product pH, a_w, moisture content, etc. Other tests may involve microbiological analysis for indicator organisms or pathogens.

Regardless of the criterion, consideration must be given to the factors influencing the analytical results (e.g., method of sampling, transportation and storage of samples, analytical procedure, etc. (see Chaps. 9 and 10). These important factors determine the utility and reliability of criteria to differentiate acceptable from unacceptable lots. Considering the limitations of microbiological testing to ensure the safety of individual food lots (see Chaps. 6, 7 and 8), greater emphasis should be placed on the conditions under which foods are produced i.e., the application of GHP and HACCP.

4.5 Determining Acceptance by Approval of Suppliers in Business-to-Business Relationships

4.5.1 Role of FSO and PO in the Approval of a Supplier

If a FSO or a PO has been established for a commodity, the first step is to determine whether it is being met. If there is agreement on the achievement, further detailed information can be shared on the control measures that have been adopted to meet the FSO/PO either directly or via a PC. Specifically, this would involve evaluating whether the adopted control measures under the food safety management system can be expected to meet the FSO/PO consistently.

During the auditing process, the FSO/PO provides a common yardstick for evaluation (see Sect. 4.7.1). The supplier and buyer of an ingredient or food ideally share the same values with regard to safety, and the supplier considers the buyer's "risk" equal to their own. The buyer should is aware of the intended use and any PC that may be necessary to ensure safety can be mutually agreed upon. The

supplier would then incorporate the necessary control measures to meet the PC into their GHP and HACCP procedures (see Sect. 4.7.1). Ideally, this information can be used for auditing either by a mixed team of experts from both companies or by any third party auditors engaged by the buyer (see Sect. 4.7). If laboratories are involved, they would be expected to use the same reference methods or comparable methods that have been validated against the reference method using international standards, e.g., ISO16140 and participate in proficiency schemes that are consistent with international standards, e.g., ISO 17043.

In situations where a FSO, PO or PC has not been established conventional systems must be used for determining whether to accept a supplier. These include inspection and auditing of GHP and HACCP systems. Where necessary, such an approach may be supported by testing representative lots of production from the supplier to determine consistency of compliance with purchase specifications. Physical, chemical and/or microbiological tests may be used for this purpose. The number of lots that should be tested should reflect hazards and risks and the consequences of non-compliance with the criteria. When it has been determined that the supplier can reliably meet purchase specifications, then product testing can be discontinued or sharply curtailed.

In contrast to the FSO/PO, microbiological criteria are of limited value in the approval process for a potential supplier. The reason is that microbiological criteria can be applied to specific lots of product to determine their acceptability but this information provides only a snapshot, and little or no confidence for what may be produced over an extended period of time. By continued testing of incoming raw material from a supplier, it would be possible to establish a history of compliance with established criteria (see Chap. 13). However, when evaluating a new supplier, microbiological testing of current production lots would not be adequate to assess variability and, in particular, the presence of a pathogen that may occur intermittently and at low frequency. Thus, confidence in a supplier's ability to meet an FSO/PO through process control has greater value than reliance upon microbiological testing of incoming lots.

4.5.2 Approval Procedures

At each transfer point in the food chain there is normally a buyer and a supplier. Ultimately, the final buyer will be a consumer. Buyers and suppliers can be in government as well as in industry. Suppliers may be domestic or in another country.

The preferred approach to managing the safety of food is to select suppliers who can be relied upon to consistently provide ingredients or foods that meet food safety requirements. Food safety systems based on prevention are much more effective than attempting to differentiate safe from unsafe lots by microbiological testing. While there may be a role for testing certain ingredients or foods, microbiological testing should be applied cautiously and used as a supplement to other information, particularly the conditions under which the material is produced. A listing of parameters that can be used to approve suppliers appears in Table 4.1.

The net result of these activities is to develop a base of suppliers that can be consistently relied upon to provide raw materials that will be safe when used as intended. This approach requires that all parties be knowledgeable in the significant hazards that may be associated with the foods supplied. The FSO/PO concept can be an effective means to communicate the significant hazards that must be controlled to ensure consumer protection.

Many of the parameters in Table 4.1 can be verified by auditing experts to establish that the conditions under which the ingredient or food is produced are acceptable. International standards, e.g., ISO 22000 and third party standards, e.g., Global Food Safety Initiative (GFSI) benchmark schemes, cover holistic food safety management systems including GHP and HACCP. Auditing against these standards by accredited certification bodies leads to certification of food businesses. Such certification may be acknowledged by buyers for the approval of suppliers and consequently further food safety audits by the buyer may be limited to those items listed in Table 4.1 that are not part of certified standards.

Table 4.1 Parameters that can be used to assess the acceptability of a supplier

Component of the food control system	Expectation
Good hygienic practice	In place and consistent with best practice
HACCP plan	In place and designed to control significant hazards based on an analysis of risk
FSO	Process is designed and validated to meet a FSO where established
PO	Process is designed and validated to meet a PO where established
Performance criteria	Validated process(es) that meet the performance criteria
Process criteria	Process criteria incorporated into HACCP plan as critical limits
Product criteria: Organoleptic, chemical, physical and biological specifications	Meets specifications
Records	Records are complete, accurate and facilitate validation and verification

Exporter/Consignor			Certificate No.
Consignee			**TITLE**
			Name and address of issuing authority
	Port of loading		Country of origin of goods
Vessel/ Aircraft	Date of departure		
Port of discharge	Final destination (if on carriage)		
Identification, Shipping marks	No. and kind of packages	Description of goods	Quantity
Container number, Seal number			
Details of producing establishments			
Details of treatment			
Attestation			
DECLARATION			
Dated at _____ (place)			
On _____(date)			
_____ Signature of signing officer		_____ Printed name	

Fig. 4.1 Example of a certificate for the export of food and food products

Certificates also may be used for foods in international trade in the form of an export certificate. An example of a certificate appears in Fig. 4.1. In addition to the expected information about the source of the food, the mode of transportation and the quantity involved the sanitary or phytosanitary status of the food are attested to by an issuing authority. The certificate is a legal document that specifies the lot is in conformity or meets:

- the specified product standards required by the importing country or in their absence the specified product standards of the exporting country,
- provisions of bilateral or multilateral agreements between the importing and exporting countries, and
- in the absence of such provisions, the standards and requirements as agreed upon, with emphasis on the use of standards and codes of practice of the Codex Alimentarius Commission.

4.6 Factors that May Affect a Decision on Lot Acceptance

The decision to accept or reject foods upon receipt at port of entry or at a buyer's operation is influenced by a number of factors. Among the most common is prior experience and confidence in the supplier and/or supplying country for compliance with all established criteria. Another is the likely impact if an incorrect decision is made to accept a defective lot. The extent of an adverse impact will depend on the likely presence and severity of a hazard in the food and whether its intended use can result in a decrease, no change, or an increase in the hazard prior to consumption as described in detail in Chap. 8. Table 4.2 illustrates the type of information about a food lot that would impact decisions regarding acceptance based on different degrees of knowledge about the source in cases where there is no obvious defect in the food and the accompanying documentation is complete.

Extensive knowledge about a food and the control system under which it was produced, leads to a high level of confidence that the ingredient or food will meet the FSO/PO and provide the expected level of protection. In this case, it would be redundant and non-productive to sample and test the incoming material. When there is some knowledge about a food but knowledge about the control system under which it was produced is incomplete, the decision to accept the lot may be determined by the consequences of an incorrect decision. It may be prudent to test the incoming material if the chance of a food safety problem is sufficiently high and testing can be expected to provide useful information. The tests that may be performed would depend on the material and the significant hazards that may be expected to occur. In the absence of knowledge about the food or applicable control processes it is necessary to develop information for the lot. Testing should be based on the hazards that may be expected to occur. There is little choice but to place greater reliance on the use of a sampling plan that reflects potential risk as described in Chaps. 5 and 8.

Criteria of various types are commonly established for foods at different stages along the food chain as described in Sect. 4.3.1. Despite these criteria, foods are not always sampled at each step to verify compliance. In reality, the majority of foods are not sampled. The decision to sample a food lot depends not only on prior experience of the supplier but also on a wide variety of other factors such as those outlined in Table 4.3. The specific use of sampling and testing for compliance with microbiological criteria is dealt with in Chap. 5.

Table 4.2 Examples of information affecting decisions regarding food lot acceptance

Types of prior knowledge about supplier of a food lot	Ideal situation – *extensive prior knowledge about the supplier of a food lot*	Less than ideal situation – *incomplete prior knowledge about supplier of a food lot*	Uncertain situation – *little to no prior knowledge about supplier of a food lot*
Basis of control measures used	Control measures established to meet an FSO (Chap. 3) and validated	Uncertain basis of control measures with little to no validation	No knowledge of basis of control measures or validation
Approval status	Approved supplier	Within approval process but not yet approved	Not an approved supplier
Compliance history with agreed specifications	History of full compliance with all process and finished product specifications	History of compliance with finished product specifications but no history of compliance with process specifications available	No history of compliance available
Audit history of supplier	Favorable history of audit outcomes	Audit history short or incomplete	No audit history established
History of record keeping by supplier	Complete record history that is accurate and enables process verification	Incomplete record history	No record history

Table 4.3 Factors that can influence the decision whether to sample and test a lot of food

Factor influencing decision to sample and test	Condition that would increase the likelihood of sampling the lot
What is the expected outcome if the food is not sampled and is accepted?	Illness among consumers is probable
Has the food commonly been involved in foodborne illness?	The food has a recent history of causing illness
What is the severity of the hazard(s)?	The expected hazard is of high severity
Is there reason to suspect the food will not meet established criteria?	Certain lots occasionally fail established criteria
Is the food intended primarily for a vulnerable population	The food is designed and intended for high risk populations
Is the food from a country or region with endemic disease of importance to food safety.	Recent reports indicate that endemic disease has resulted in foodborne illness among consumers of an importing country.
Is the country or supplier known to exercise control over the production of the food?	The exporting country's inspection program is considered inadequate for the expected hazard(s) in the food.
Can a sampling plan be used to detect unacceptable lots, particularly when a small number of defective units are expected?	When defective lots occur the number of defective units is sufficiently high to be detected.
Can the sampling plan detect a low prevalence of a pathogen of concern?	When present, the pathogen occurs in sufficiently high numbers that it can be detected by the method employed.
What is the expected complexity, accuracy, sensitivity and time for the result?	The laboratory method is easily performed, accurate, sensitive and the time to obtain a result will not lead to a decrease in the quality of the product.
Does the laboratory have the equipment and expertise to analyze the samples?	Yes
Where is the food located; can the lot be easily sampled?	The lot is located nearby and can be easily sampled.
Ease of transporting the samples to the laboratory	The laboratory is situated locally.
What is the cost of the product that will be sampled,	The product samples need not be purchased.
Are there sufficient funds, personnel and laboratory support to collect and analyze the samples.	These resources are available.
Are there any outside influences to consider (e.g. regulatory standards, supplier specifications)	There are no outside influences.

To respond differently to the questions in Table 4.3 would decrease the likelihood that a food lot would be sampled and tested. Thus, foods believed to be of low risk to consumers would be sampled infrequently or not at all. However, it is important to maintain constant vigilance in regard to food lots delivered to a food business or arriving at the port of entry. Any changes to the information available, e.g., an unfavorable audit report or an outbreak of foodborne illness relevant to the type of food being delivered should trigger changes to decisions regarding sampling frequency and testing approach. A reduced or tightened inspection approach may be warranted under such circumstances (see Chap. 11).

4.7 Auditing Food Operations for Supplier Acceptance

In the context of food production, auditing is a systematic process of objectively obtaining and evaluating evidence as a means of verifying compliance against a pre-determined scheme. Schemes can be international standards like ISO 22000, GFSI benchmarked schemes, quality assurance schemes issued by trade bodies or proprietary supplier specifications. As discussed earlier, supplier audits

typically cover more than just food safety management systems and often extend into quality systems, general management systems and commercial systems. Food companies engaged in supplier acceptance audit activities should ensure the integrity of their activities by following internationally accepted standards for the conduct of audits.

Standardization of audit activities has developed considerably over the last decade. There are now two key international standards that provide an audit framework and establish consistent standards for bodies involved in audit. ISO 19011:2012 (ISO 2011a) provides guidelines for auditing management systems. It is aimed at companies engaged in audits, including smaller food businesses, and outlines how management system audits should be conducted. The standard concentrates on internal audits (termed first party audits) and supplier audits (termed second party audits) whilst third party audits by external certification bodies against standards for certification purposes are further elaborated in a complementary standard called ISO/IEC 17021:2011 (ISO 2011b). The details included in this latter standard will not be elaborated in this chapter.

In brief overview, ISO 19011 establishes a set of principles designed to ensure that an audit is reliable and effective and can be used by the audited company as a basis for improvement. The principles are as follows:

- Integrity of the auditors (professionalism and competency)
- Fair presentation (truthful and accurate reporting)
- Due professional care (reasoned judgement)
- Confidentiality (security of information)
- Independence (impartiality and objectivity)
- Evidence-based approach (verifiable audit evidence)

The audit program itself, consisting of one or more audits, is broken down into a cyclical activity involving four main components called Plan-Do-Check-Act. The planning stage involves establishing objectives for the audit program and developing the details of the audit program which in turn includes the personnel and audit procedures. The implementation stage details the activities involved in actual audit(s) undertaken as part of the audit program. The monitoring stage is where an overview of progress is maintained including issues such as performance of the audit team members and stakeholder feedback. Finally, these stages are followed by a review of the audit program and any adjustments necessary to improve the process prior to starting a further program cycle.

Food companies that use standardized audit procedures as a basis for supplier audits are more likely to ensure consistency, transparency and fairness in their audit activities which provides a sound basis for good supplier relations and development of the supplier base.

4.7.1 Further Considerations for Auditing a Supplier When a FSO/PO Has Been Established

If a food business is producing or selling a food that is subject to a FSO or a PO it may need to establish a PO of its own as an acceptance criteria for raw materials produced by a supplier. For example, a producer of dried milk used as an ingredient in a dry mixed infant formula will have to assure that the level of *Salmonella* is below a certain level. If a PO for infant formula was established as <1 *Salmonella* /10^8 g of product, then the PO for the milk powder ingredient could be set ten times lower, for example, < 1 *Salmonella* /10^9 g of product. If the initial concentration of *Salmonella* in milk was 1 colony forming unit (cfu)/10 ml (−1.0 log cfu/ml) meeting such a PO would require treatment of the milk to achieve an 8 log reduction in *Salmonella*. The 8-log reduction is an example of a PC that could be set by the supplier in order to meet the buyer's PO. The PC could be achieved by pasteurization at a temperature of 75 °C for 8 s as a minimum before spray drying or by another means (see Chap. 15).

This process criterion would be documented as a critical limit for the thermal process CCP in the supplier's HACCP plan. Recontamination should also be prevented through the application of GHP. For some GHPs (e.g., air filtration) a process criterion could be set, but for others (e.g., dry cleaning practices) criteria cannot easily be established and monitored.

Chapter 3 contains information on how control measures should be set to meet FSOs. Auditors should take account of this information when developing their auditing procedures as the basis for evaluating whether the operation being audited has established effective risk-based controls that can be justified. For example, will the system meet established PC? Can the critical limits at CCPs (i.e., process criteria and product criteria) be expected to prevent, eliminate, or reduce the hazard(s) to acceptable levels? If default criteria are selected as a basis for control, are they being correctly applied?

During a supplier audit when a PO for a product has been established by the buyer or a regulator it is essential that there is an objective evaluation of evidence regarding the validation of any risk management metrics established by the supplier and designed to meet the PO such as PC, process or product criteria. This can be done during the pre-audit assessment process by examination of any relevant validation records and supporting documents. This should be carried out by a member of the audit team who has the appropriate microbiological skills. Verification that these critical criteria are met consistently can commence in the pre-audit phase by examination of monitoring records, but would need to be completed during the physical audit.

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Chapter 5
Establishment of Microbiological Criteria

5.1 Introduction

Internationally recognized principles for the establishment of microbiological criteria (MC) are established by the Codex Alimentarius Commission (CAC 2013). These principles were initially developed through World Health Organization (WHO)/Food and Agriculture Organization (FAO) consultations (Christian 1983) and have evolved through a number of revisions, each with input from the ICMSF. An MC represents one form of the acceptance criteria discussed in the previous chapter.

The establishment of MC for a food requires knowledge of the relevant microorganisms and their behavior and occurrence in the food. It is desirable that MC should be established with an explicit link to a quantified public health improvement via a microbiological risk assessment. However, it is also recognized that there is still a place for MC based on an unquantifiable but implicit public health impact via hazard reduction as well as MC based on empirical knowledge of hygiene and what can be achieved through application of accepted good hygienic practices.

The desire to establish MC based on a quantifiable risk reduction may be fulfilled by the use of the risk management metrics of FSO and PO to translate the findings of a risk assessment into control parameters that can be achieved by the food industry. An example is the MC for *Cronobacter* spp. in powdered infant formula developed through the Codex Alimentarius Commission (CAC 2009). However, few FSOs and POs have been established, and consequently MC established on this risk basis are not yet in broad use. The traditional types of MC are more frequently used by governments and industry to ensure food safety and hygiene. Unfortunately, their basis is often less transparently linked to improvements in public health and thus varying interpretations may directly lead to non-tariff trade barriers and add to the burden of irrelevant specifications between trade partners. According to the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) (WTO 1995), every criterion that has not been established through the application of Codex principles can be challenged when its application leads to a trade barrier.

As discussed in Chap. 2, an FSO is a statement of the maximum frequency and/or concentration of a microbiological hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP) and a PO is a similar concept that applies at an earlier step in the food supply chain to contribute to meeting the FSO or ALOP. If an FSO/PO exists for a food, it is important

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Table 5.1 Characteristics of microbiological Food Safety Objectives/Performance Objectives (FSO/PO) and microbiological criteria (MC)

FSO/PO	MC
A goal upon which food processes can be designed so the resulting food will be acceptable	A statement that defines acceptability of a food product or lot of food
Applied to food processing operations	Applied to individual lots or consignments of food
Components: Maximum frequency or concentration of a microbiological hazard	Components: Microorganism of concern and/or their toxins/metabolites Sampling plan Analytical unit Analytical method Microbiological limits Number of analytical units that must conform to the limits
Can be used to establish microbiological criteria	Cannot be used to establish a FSO
Used only for food safety	Used for food safety or quality characteristics
Based on what risk managers believe will ensure the safety of a food	Based on a FSO, a PO or what risk managers believe will ensure a food to be safe or acceptable for the intended use
Can be used to drive change in processing conditions for a food commodity and improve its safety	Can be used to drive change in processing conditions so individual lots or consignments will meet established criteria
Very suitable for assessing the food safety system of a food operation	Not very suitable for assessing a food safety system of a food operation. Sampling lots of food from an operation provides a snapshot of the level of control at the time of collection but provides little confidence for past or future lots.
A high level of confidence is possible when processes are designed and validated to meet a FSO or PO	Confidence may be less certain if a FSO or PO is not used when processes are designed and validated to meet a MC

that MCs are compatible with the articulated value. MC should not be so lenient that the intended public health goals cannot be achieved. Conversely, an MC that is excessively stringent relative to an FSO/PO may result in rejection of food even though it has been produced under conditions that provide an acceptable level of protection. As illustrated in Table 5.1, FSOs/POs and MC differ considerably in function and content.

In the Codex Alimentarius Commission, the use of MC in environmental monitoring is not included in the scope of the guidance document (CAC 2013). In keeping with this approach, this chapter will similarly exclude the establishment of MC for environmental monitoring. This will be dealt with in Chap. 12.

5.2 Purposes and Application of Microbiological Criteria for Foods

Developing meaningful MC for a food is a complex process that requires considerable effort and resources. Therefore, MC should be established only when there is a need and when it can be shown that their application will be effective for the protection of public health and practical to implement and/or enforce. The microorganism subject to an MC should be relevant to the stated purpose and the MC should be established at a particular point in the food supply chain. According to Codex (CAC 2013), there are multiple purposes for establishing MCs, including:

- "Evaluating a specific lot of food to determine its acceptance or rejection, in particular if its history is unknown."
- "Verifying the performance of a food safety control system or its elements along the food chain, e.g., prerequisite programs and/or HACCP systems."

- "Verifying the microbiological status of foods in relation to acceptance criteria specified between food business operators."
- "Verifying that the selected control measures are meeting POs and/or FSOs."
- "Providing information to food business operators on microbiological levels, which should be achieved when applying best practices."

5.3 Definition of Microbiological Criterion

The Codex Alimentarius Commission defines an MC as follows:

*"A **microbiological criterion** is a risk management metric which indicates the acceptability of a food, or the performance of either a process or a food safety control system following the outcome of sampling and testing for microorganisms, their toxins/metabolites or markers associated with pathogenicity or other traits at a specified point of the food chain."* (CAC 2013).

5.4 Types of Microbiological Criteria

Chapter 4 recognized three types of general acceptance criteria for food lots. These are standards, guidelines and specifications (NRC 1985). In the context of MC, these can be elaborated as follows:

- *Microbiological standard* – a mandatory criterion that is incorporated into a law or ordinance
- *Microbiological guideline* – an advisory criterion used to inform food operators and others of the microbial content that can be expected in a food when best practices are applied
- *Microbiological specification* – part of a purchasing agreement between a buyer and supplier of a food; such criteria may be mandatory or advisory according to use

5.4.1 Microbiological Standards

Microbiological standards are established by regulatory authorities and generally define the microbiological profile that foods must meet to be in compliance with a regulation or policy. Foods not meeting a standard are in non-compliance and would be subject to removal from the marketplace or required actions to bring them into compliance. Standards may be established for a wide variety of reasons, but are best applied when risk is sufficiently high and compliance is essential for public health protection. Microbiological standards should apply to those foods and/or points of the food supply chain where no other more effective tools are available and where they are expected to improve the degree of consumer protection and/or facilitate fair trade. Ideally, the standards should be based on a tolerable level of risk communicated via a FSO or PO for the hazard of concern. Microbiological standards may be used by both industry and government. Microbiological standards established for lot acceptance should be applied equally to domestic and imported foods.

5.4.2 Microbiological Guidelines

Microbiological guidelines may be established by many players, (e.g., regulatory authority, industry trade association, food company). They indicate what is expected for the microbial profile of a food produced under a food safety control system or its individual elements like GHP. Food operators use

microbiological guidelines as a basis to design their control systems. Guidelines are advisory in nature and may not lead to rejection of a food. Microbiological guidelines may be a preferred means to inform and direct food businesses within a segment of the industry to improve its practices, particularly when insufficient data prevent the establishment of a microbiological standard. A microbiological guideline alone, along with guidance material on what constitutes best practices, may be adequate to bring about the changes necessary to improve food safety and quality. This may negate the need to establish a standard.

5.4.3 Microbiological Specifications

Buyers establish purchase specifications to reduce the likelihood of accepting an ingredient or a finished food that may be unacceptable in terms of safety or quality. For example, microbiological specifications often define the microbiological limits for an ingredient, so that when it is used, the final product will meet all requirements for safety and quality. It is common practice for buyers along the food supply chain to establish microbiological specifications for the materials they purchase. In most cases, the specifications are advisory unless contractually mandated. Often the materials are sampled only on an as-needed-basis. In other cases (e.g., sensitive ingredients), each incoming lot may be tested either by the receiving company or through a "Certificate of Analysis" (COA) by the supplier.

5.5 Application of Microbiological Criteria

5.5.1 Application by Regulatory Authorities

Regulatory bodies may establish MC in the form of microbiological standards or guidelines as appropriate. Microbiological standards are the preferred acceptance criteria applied to trigger mandatory action by the food business. Some are applied to lot acceptance at a specified point in the food supply chain, e.g., port of entry or foods in commerce, whereas others are applied at intermediate processing stages and are used to indicate that a given process is being operated in accordance with good hygienic practice. In the former case, examples of mandatory action resulting from non-compliance could be lot rejection, food recall or reprocessing. Whereas in the latter case of standards applied to intermediate processing steps in the food supply chain, the mandatory action is rarely removal of the food from the market, but more often a requirement on the food business to make improvements to their hygiene procedures.

Regulatory authorities apply microbiological standards in the context of their official food controls by independently testing foods against the standards and/or by inspecting the testing results achieved by food businesses and maintained in their records for verification of compliance.

Microbiological guidelines on food safety are commonly introduced by regulatory authorities in situations where insufficient data are available to justify the establishment of a microbiological standard, or as an interim communication to food businesses on an acceptable microbiological profile for foods in trade prior to the introduction of a microbiological safety standard, which generally takes significantly longer due to the legislative process. In contrast, microbiological guidelines governing food quality that are issued by regulatory authorities rarely progress to microbiological standards. Ideally, microbiological guidelines should be issued by regulatory authorities in consultation with the affected food industry and should be supplemented with sufficient guidance to help with compliance.

Regulatory authorities encourage food businesses to adopt microbiological guidelines either directly or by facilitating their inclusion in quality assurance schemes. However, compliance with MC in the form of guidelines generally cannot be enforced, as they usually lack the legal basis to do so.

5.5.2 Application by Food Businesses

A food business can enter into an agreement with a supplier to ensure that food being purchased will meet mutually agreed upon MC (see Sect. 5.4.3). Food operators frequently establish purchase specifications for food ingredients and other materials to help ensure compliance with microbiological standards and as a means to help ensure product quality. The factors previously outlined in Table 4.2 can influence whether a buyer will sample ingredients or foods upon receipt. For example, an audit of an operation may suggest that a supplier cannot consistently meet purchase specifications. This may lead the buyer to sample incoming lots of purchased material until a follow-up audit indicates improvement. Lots that fail to meet the established specifications may be rejected.

In addition to checking compliance with regulatory standards (see Sects. 5.4.1 and 5.5.1), MC may be applied by food businesses to formulate design requirements and to examine products as one of the measures to verify the efficacy of their food safety control system or one of its elements, i.e., GHP and HACCP. Such criteria will be specific for the product and the stage in a process or in the food supply chain at which they apply. They may be stricter than the criteria used for regulatory purposes.

5.5.2.1 Application in GHP

MC can be used to check certain aspects of GHP. Examples include verifying the acceptability of water if not supplied by a source tested by others, and microbial limits that are to be met when the cleaning and disinfecting routines are properly performed. These criteria often consist of aerobic counts or indicator microorganisms and reflect experience of what is attainable with the equipment, materials and conditions that exist for the operation. Another option to assess GHP is to sample product at selected times and steps in an operation and analyze for aerobic counts or other indicators. For example, ICMSF (2011) recommended the use of aerobic colony counts and Enterobacteriaceae as indicators for verification of adherence to GHP and processing control in pasteurized egg products. Evidence of an increase in the microbial concentration on or in a product may be due to microorganisms acquired from contact with equipment as it is being processed. In-process criteria should be based on knowledge of the conditions that influence microbial content during processing. It should also be recognized that non-microbial methods may also be useful for verification of GHP. For example, adenosine tri-phosphate (ATP) testing provides a rapid result and can be useful for verifying effectiveness of cleaning for many surfaces.

5.5.2.2 Application in HACCP

MC can be used to verify the performance of a HACCP plan, but are not useful for verifying control at an individual process step because the result from testing against MC reflects the combined effect of all proceeding control steps in the production process rather than the effect of a single process step. Also, MC are not suitable for monitoring critical control points (CCPs) as defined in Hazard Analysis and Critical Control Point System and Guidelines for its Application (CAC 2003b). Monitoring procedures must be able to detect loss of control at a CCP, thus monitoring should provide this information in time for corrective actions to be taken to regain control before there is a need to reject the product. Consequently, on-line measurements of physical and chemical parameters are often

preferred to microbiological testing because results are often available more rapidly and at the production site.

Microbiological testing in its broadest sense can play an important role in validation of critical limits associated with CCPs, but this is not typically handled through the use of MC. Validation of critical limits of CCPs should be done prior to implementation in a production process or after changes have been made in the production process. Approaches used for validation of control measures are detailed by the Codex Alimentarius Commission (CAC 2008) and Zwietering et al. (2010). Typically, such validation would be achieved by a number of complimentary approaches like literature review and modeling that includes, where necessary, experimental microbiological approaches. Here, the concentration of microorganisms can be standardized prior to the process step being validated, which allows accurate estimation of the control achieved by that step under a certain set of operating parameters. An effective combination of processing parameters that achieves the PC for that step (if a PC has been established) may be adopted as critical limits for that process step – MCs are not suitable for this purpose.

5.6 Principles for the Establishment of Microbiological Criteria

The safety of foods is principally assured by control at the source, product design and process control, and the application of GHP during production, processing, labeling, handling, distribution, storage, sale, preparation and use, in conjunction with the application of the HACCP system. When based on valid scientific considerations, this preventive approach offers more control than microbiological testing because the effectiveness of microbiological examination to assess the safety of foods is limited due to sampling probability, day-to-day variation, limitations of test methods and other factors. However, MCs may be useful to provide verification that controls are working as intended.

A scientific basis should be used for developing MCs and, where sufficient data are available, they should be based on a risk assessment appropriate to the foodstuff and its use. Transparency in the development of MC is also important to assist in communicating the public health relevance of the criterion to diverse stakeholders and to meet the requirements of fair trade. Periodic review of MCs in respect to emerging pathogens, changing technologies and new understandings of science is important.

An MC should be established only when there is a definite need and its application is practical. Such need may be demonstrated by epidemiological evidence that the food under consideration may represent a public health risk and that a new MC can make a meaningful impact on improving consumer protection. A risk assessment may provide further evidence to support establishment of an MC. An MC should be established only when it is technically attainable by applying GHP and HACCP.

When establishing an MC, consideration should be given to:

- evidence of actual or potential hazards to health
- microbiological status of the raw materials
- effect of processing on the microbiological status of the food
- likelihood and consequences of microbial contamination and/or growth during subsequent handling, storage and use
- intended use of the food
- the consumers concerned, including relevant sub-populations and consumption habits
- cost/benefit ratio associated with the application of the criterion
- the likelihood of detection of the microorganism of concern

The intended use is an important consideration. Food safety is defined as "assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use" (CAC 2003a). Thus, a raw agricultural commodity that is intended to be thoroughly cooked before consumption could contain *Salmonella* and with adequate processing, not cause harm. A stringent sampling plan for *Salmonella* in such a food would normally have no value. Proper labeling with adequate instructions for preparation and use could be more effective.

Consideration of the intended use should also include who is going to prepare the product (e.g., professional caterers, homemakers, children) and the group of consumers for which the food is intended. Babies, the elderly, immuno-suppressed individuals, etc., are more vulnerable, for example, than healthy adults, thus, greater care is essential for the production or preparation of food specifically intended for these consumers. This should be reflected in the stringency of MC and accompanying sampling plans.

Consideration of cost/benefit should assess whether establishing and enforcing a criterion would be an effective means of using the available resources to improve public health. If no health benefit is likely to result, implementation of a new criterion should be questioned. Additionally, the criteria should be administratively feasible by the regulatory authorities. These considerations fall under the activity of choosing an adequate risk management option within the risk management concept, where other options will be considered as well.

5.7 Components of Microbiological Criteria for Foods

According to the Codex Alimentarius Commission (CAC 2013) an MC consists of:

- the purpose of the MC
- the food or process to which the MC applies
- the specified point in the food supply chain where the MC applies
- the microorganism(s) and the reason for their selection
- the microbiological limits (m, M) and/or other limits considered appropriate to the food
- a sampling plan defining the number of samples to be taken (n), the size of the analytical unit and where appropriate, the acceptance number (c)
- depending on its purpose, an indication of the statistical performance of the sampling plan
- analytical methods and their performance parameters
- action to be taken when the MC is not met

When applying an MC for assessing products, it is essential, in order to make the best use of money and resources, that only appropriate tests be applied to those foods and at those points in the food supply chain that offer maximum benefit in providing consumers with foods that are safe and suitable for consumption. The MC should be economically feasible for food businesses to execute. Consideration also needs to be given to sampling; particularly the type of sample, sampling strategy (e.g., random, stratified) and the sampling frequency.

5.7.1 Microorganisms and Their Toxins/Metabolites of Importance in a Particular Food

Microorganisms and/or their toxins/metabolites of concern include:

- bacteria, viruses, yeasts, molds and algae;
- parasitic protozoa and helminths; and
- microbial toxins/metabolites.

According to the Codex Alimentarius Commission, this includes the markers of microbiological pathogenicity (e.g., virulence-related genes or plasmids) or other traits (e.g., anti-microbial resistance genes) where/when linked to the presence of viable cells (CAC 2013).

The microorganisms included in a specific MC should be widely accepted as relevant to the particular food and/or process technology, either as pathogens, as indicator organisms or as spoilage microorganisms. Microorganisms whose significance in the specified food is doubtful should not be included in an MC. For example, the mere finding, with a presence-absence test, of certain microorganisms known to cause foodborne illness (e.g., *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus* and *Vibrio parahaemolyticus*) does not necessarily indicate a threat to public health. These bacteria typically require growth in a food before they are considered a significant hazard.

Where pathogens can be detected directly and reliably (e.g., performance of sampling plans), consideration should be given to testing for them in preference to testing either directly or indirectly for indicator organisms. Testing for an indicator microorganism may be useful as discussed below.

5.7.1.1 The Use of Indicator Organisms

Indicator organisms are frequently used to examine foods or ingredients. An important use of indictors is to verify process control and identify opportunities for process improvements. Most of the considerations used to set MC are applicable for indicators; however, indicators are not used solely for pathogenic concerns. Microorganisms, their cellular components, or their metabolic products used as indicators may indicate:

- the possible presence of a pathogen or toxin (e.g., *S. aureus* for potential enterotoxin and/or excessive human handling in cooked crab meat)
- the possibility that faulty practices occurred during production, processing, storage and/or distribution (e.g., Enterobacteriaceae in pasteurized milk)
- the suitability of a food or ingredient for a desired purpose (e.g., *Escherichia coli* in nuts for ice cream)
- an estimate of the keeping quality of perishable foods during the expected conditions of handling and storage (e.g., yeast in yogurt)
- the possibility of changes in the food through fungal activity that would result in a less acidic food, thus making the food potentially more hazardous
- the effectiveness of cleaning and disinfection (e.g., ATP for residual soil)

Indicator microorganisms and agents can be divided into indicators of potential human contamination, fecal contamination, survival of a pathogen or spoilage organism, or post-processing contamination. Examples of microbial indicators that can be analyzed quantitatively or qualitatively include aerobic bacteria, coliforms, Enterobacteriaceae, *E. coli*, yeasts, molds, proteolytic bacteria and thermophilic bacteria. Examples of cellular components that can be used include ATP, ribonucleic acid (RNA), endotoxins (e.g., limulus lysate test for cellular polysaccharides) and various enzymes (e.g., thermonuclease). Examples of metabolic products used as indicators include hydrogen sulfide (early putrefaction), carbon dioxide (spoilage by *Zygosaccharomyces bailii*), lactic acid (certain meat products including ham), ethanol (in fruit juice), diacetyl (in fruit juice or beer) and ergosterol (mold in grain).

Some of the characteristics of an ideal indicator organism include the following:

- presence indicates potential for spoilage, faulty practice or faulty process
- easily detected and/or quantified
- survival or stability (including inactivation kinetics) similar to or greater than the hazard or spoilage microorganism

- growth capabilities (requirements) similar or faster than the hazard or spoilage microorganism
- identifiable characteristics of indicator are stable
- methods are rapid, inexpensive, reliable, sensitive, validated and verified with positive control
- quantitative results have a correlation between indicator concentration and level of hazard or spoilage microorganism
- results are applicable to process control

It must be recognized that indicators often represent a compromise that is less than the ideal of being able to test for the microorganism(s) or toxin(s) of concern. However, indicators offer considerable advantages that ensure their continued use. To fulfill their purpose as indicators it is essential to select indicators that provide the best information for the intended purpose with the least amount of compromise.

In practice, indicators seldom, if ever, prove the presence or the absence of a target microorganism; they merely indicate the possibility. For purposes of process control, the absence or low numbers of an indicator can verify that a process is under control, thus there is a lower likelihood that the unacceptable target microorganism is present. However, a pathogen may be present independent of an indicator. Thus, combining indicator tests that provide information on conditions that reduce the likelihood of the occurrence of a pathogen with periodic verification testing for the pathogen is appropriate in many situations. Indicators can be very useful, but their selection and application must be done with care and a thorough understanding of how to accurately interpret the analytical results.

5.7.2 Microbiological Limits

Microbiological limits may be established as a basis to assess the safety or quality of a food and should be compatible with any POs that have been established for a specific food. Limits should be based on microbiological data appropriate to the food and should be applicable to a variety of similar products. The process of establishing limits for use as standards should include collecting and analyzing data from a variety of operations to determine what can be expected for foods produced under acceptable conditions of GHP and HACCP. These data can then be used to establish limits that can be met by all who operate under acceptable conditions. Alternatively, the limit can be made more stringent if improvement is deemed necessary in a certain segment of industry to reduce the likelihood of a hazard. This assumes operators can adapt by making practical modifications. If, however, the technology does not exist or is not affordable, then the more stringent limit will fail and the desired improvement will not be achieved. The process of establishing MC, and other acceptance criteria, should be transparent and allow input from all interested parties.

Microbiological limits relate only to the specified time and place of sampling and not to the presumed number of microorganisms at an earlier or a later stage. Because GHP aims at producing foods with microbiological characteristics significantly better than those required by public health considerations, a numerical limit in a guideline may be more stringent than in a standard or end product specification.

In the establishment of microbiological limits, any changes in the microbiota likely to occur during storage and distribution (e.g., decrease or increase in the numbers) should be taken into account. The risk associated with the microorganisms and the conditions under which the food is expected to be handled and consumed should also be considered. These considerations are discussed in Chap. 8. Microbiological limits should also take account of the likelihood of uneven distribution of microorganisms in the food (see Chaps. 6 and 7) and the inherent variability of the analytical procedure (see Chap. 9).

Whether foods are acceptable or not is defined in a criterion by the:

- microbiological limit(s)
- number of samples examined
- size of the analytical unit
- number of units that should conform to the limits

If a criterion requires the absence of a particular microorganism, the number and size of each analytical unit should be indicated. It should be recognized that no feasible sampling plan can ensure the absence of a particular microorganism in the entire contents of the lot.

The microbial population in many foods produced under GHP and HACCP is generally not explicitly expressed because they are as low as is reasonably achievable. In some cases, the levels cannot be measured due to the technical problems involved. For instance, a lot of canned food that has received a "bot cook" will most probably not contain surviving spores of *C. botulinum* in 10^{10} or 10^{11}/g of product. Likewise, enteric pathogens are not likely to be found in many kilograms of pasteurized products. Setting limits for pathogens in processed foods in which a validated kill step is included during processing should not be an arbitrary activity, but should be done only if there is a need to detect contaminated product.

Limits for use in purchase specifications are best established from data collected during normal production when the operation is under control. It is not uncommon for a company to establish more stringent criteria for its own use to ensure compliance with customer and regulatory requirements.

In sampling by attributes procedures, microbiological limits, *m* and *M*, define the presence/absence or concentration of a microorganism, microbial toxin or metabolite that differentiates acceptable from unacceptable sample units of food, and *c* is the maximum allowable number of defective or marginally acceptable sample units (see Chap. 7). In a two-class plan, *m* separates acceptable units from defective units, while in a three-class plan; *m* separates acceptable units from marginally acceptable units. The limit *m* may be considered by those establishing the criterion to be acceptable and attainable through application of GHP and/or HACCP. In a three-class plan, *M* separates marginally acceptable units from unacceptable units (see Chap. 7).

In sampling procedures not based on attributes, the microbiological limits (*m* and *M*) are replaced by alternative limits. For example, for variables plans, the acceptable microbiological quality limit (*V*) and the maximum proportion (*p0*) of the lot that can be accepted with concentrations above the limit (*V*) (see Chap. 7).

5.7.3 Sampling Plans, Sampling Procedures and Handling of Samples Prior to Analysis

Sampling plans should include the sampling procedure and the decision criteria to be applied to the result, based on examination of a prescribed number of sample units and subsequent analytical units of a stated size by defined methods. A well designed sampling plan defines the probability of detecting microorganisms in a lot, but no sampling plan can ensure the absence of a particular microorganism from an entire lot. Sampling plans should be administratively and economically feasible.

In particular, the choice of sampling plans should take into account the:

- risks to public health associated with the hazard (severity and likelihood of occurrence of the hazard)
- susceptibility of the target group of consumers (very young or old, immune-compromised, etc.)
- heterogeneity of distribution of microorganisms where variables sampling plans are employed
- randomness of sampling

- acceptable quality/safety level (i.e., percentage of non-conforming or defective sample units tolerated)
- desired statistical probability of accepting or rejecting a non-conforming lot

The information needed for the first two points could be obtained through a risk assessment; however, good epidemiological data may suffice. For many applications, two- or three-class attribute sampling plans may be useful. In general, the greater the risk, the more stringent (e.g., greater number of samples) should be the sampling plan. For a detailed discussion of establishing sampling plans, see Chaps. 6, 7 and 8.

The time between taking the field samples and analysis should be as short as reasonably possible and the conditions during transport to the laboratory (e.g., temperature) should not allow an increase or decrease in the numbers of the target microorganisms. By controlling these conditions the results should reflect, within the limitations given by the sampling plan, the microbiological conditions of the food. For samples taken by regulatory authorities as part of official controls, consideration should be given to maintaining the chain of evidence by ensuring that the integrity and identity of the sample is maintained and the handling procedure is recorded. For further details see Chap. 9.

5.7.4 Microbiological Methods

The choice of microbiological method can have a significant impact on the quantitative and qualitative results generated. Accordingly, MC must specify the method used. The microbiological methods specified should be reasonable with regard to complexity, availability of media and equipment, ease of interpretation, time required and costs. Where possible, only methods for which the reliability (e.g., specificity, sensitivity, reproducibility) has been statistically established by comparative or collaborative studies in several laboratories, i.e., validated methods, should be used. Reference methods established by international standards organizations like ISO, AOAC and CEN fit these criteria. Moreover, preference should be given to methods that have been validated for the commodity of concern. Alternative methods to reference methods like rapid methods, can be used, providing that they have been validated against a reference method in keeping with an internationally recognized validation standard (e.g., ISO 16140). Additional information on methods and their reliability is provided in Chaps. 9 and 10.

5.7.5 Reporting

The test report should provide complete information to identify the sample, the sampling plan, the test method and, if appropriate, the interpretation of the results.

5.8 Example of Microbiological Criteria for Egg Products

Table 5.2 provides an example of ICMSF (2011) recommended MC for certain egg products that might result from application of the principles described in this chapter. The criteria include indicators (i.e., aerobic colony count and Enterobacteriaceae) as well as a pathogen (*Salmonella*), methods of analysis, number of samples to be collected (n), number of samples that must conform to the criteria (c), and the microbiological limits (m and M). This brief summary must be supplemented with additional information such as a statement for why these criteria were selected and are considered necessary; the step(s) in the food supply chain where the criteria are to be applied; method of sample collection, handling and

Table 5.2 Example of microbiological criteria for pasteurized liquid, frozen, and dried egg products

Microorganism	Analytical method[a]	Case	Sampling plan & limits/g			
			n	c	m	M
Aerobic colony count[b]	ISO 4833	2	5	2	10^3	10^4
Enterobacteriaceae	ISO 21528-2	5	5	2	10	10^2
			Sampling plan & limits/25 g			
			n	c	m	M
Salmonella	ISO 6579	10[c]	5[d]	0	0	–
		12[c]	20[d]	0	0	–

Adapted from ICMSF (2011)
[a]Alternative methods may be used when validated against ISO methods
[b]Aerobic colony count not recommended for egg albumin
[c]Case 10 for products to be cooked, case 12 for RTE applications with potential for abuse
[d]Individual 25 g analytical units

preparation for analysis; the analytical unit (in this example the unit for the *Salmonella* analysis is 25 g); whether the analytical units can be composited for analysis (in this example the five 25 g analytical units could be combined into a single 125 g composite for analysis); and disposition of lots that do not meet the criteria. The criteria can be made more stringent if the eggs are intended for a sensitive population (e.g., hospitals, institutions for the elderly).

Alternative approaches may be used to develop national standards. Because national standards are mandatory requirements that must be met by industry, they may provide more specific information regarding where the criteria apply in the food supply chain and actions to take when non-conforming product is found. As discussed in Sects. 5.4 and 5.5, national standards may be less strict than guidelines and specifications. For example, European Commission (EC 2007) standards for egg products do not include standards for an aerobic colony count in egg products; however, EC (2007) and ICMSF (2011) Enterobacteriaceae criteria for pasteurized liquid, frozen and dry egg products have the same values for *n*, *c*, *m* and *M*. The EC standard also states that the standard applies at the end of the manufacturing process, and that the efficacy of the heat treatment and controls to prevent recontamination should be checked in situations where non-compliance is detected. Further, the EC (2007) *Salmonella* standard applies to "Egg products, excluding products where the manufacturing process or the composition of the product will eliminate the salmonella risk." Thus a company with a validated HACCP plan to control *Salmonella* may choose to test the product for verification purposes, but is not required by the government to use a specified sampling plan.

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Chapter 6
Concepts of Probability and Principles of Sampling

6.1 Introduction

Management of food safety using the approaches outlined in the first five chapters, based on controlling hazards through Good Hygienic Practices (GHP) and the Hazard Analysis Critical Control Point (HACCP) strategy, is much more effective than trying to ensure safety through end-product testing. Nonetheless, end-product testing is useful to verify that the food safety management system is working effectively or to indicate when the status of a lot is in doubt. This chapter discusses the concepts of probability and sampling that, on the one hand, show the practical limitations of end-product testing and, on the other hand, form the basis of the rational design of statistically-based sampling plans (see Chap. 7), including ICMSF's 15 cases (see Chap. 8).

In Chaps. 6 and 7 it is assumed that the test used to assess a microbiological attribute of a sample is completely accurate, i.e., that if the organism of interest is present in the sample or present at a level that exceeds the criterion, it will be detected and the test result will always be "positive". Conversely it is assumed that if the sample does not contain any viable cells of the organism of interest (or they are present at a level lower than the tolerable level) the result of the test will always be "negative". Often these assumptions are not valid and further issues concerning sampling, sample transport and preparation for analysis and the imprecision of microbiological methods are addressed in Chaps. 9, 10 and elsewhere in the text.

6.2 Probability

Consider a trial or a test which has an uncertain outcome, such as a test for whether a specific organism of interest is present in a food. When applied to a sample of that food the test is either positive or negative, indicating the presence of the organism only if it is present and detected in the sample (positive). If many of the organisms were present in the food, we would expect many sample units to yield a positive result if tested. If only a few organisms were present, however, we might expect that fewer sample units would yield a positive result. In the two cases, the probability of a positive result would be respectively high and low.

The probability of a positive result is, in fact, the *long-run proportion* of times a positive result occurs out of all the times we test the food. Thus, if a positive result occurs 112 times in 1000 tests, we estimate the proportion of defective units, or the probability of encountering a defective unit to be 112/1000 = 0.112, while if it occurs 914 times in 1000 trials, we estimate the probability to be 914/1000 = 0.914. The word "estimate" is used because, if we were to run 1000 trials again on the

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Microorganisms in Foods 7, https://doi.org/10.1007/978-3-319-68460-4_6

same material and using the same procedure, we could not be sure of again observing exactly 112 and 914 positive results, respectively. The results, however, should be close to this proportion because 1000 is a large number of trials and random effects during sampling will be relatively minor. Thus, the *estimated* probability of a positive result is the proportion of times a positive outcome occurs among the trials or tests actually made. A probability can only take values from 0 to 1. It will be zero if the organism is absent from the food (within our assumption that the test procedure will reliably detect any organism present) and one if every unit of the food contains the organisms at an unacceptable level.

What does an observed proportion of trials such as 0.112 signify? Suppose we divided the *entire* lot of food into small sample units, say perhaps 10,000,000 one-gram units, and then went through the test procedure on each of these sample units. Suppose 1,051,200 gave positive results. Then the ratio 1,051,200/10,000,000 (the actual proportion of positives) = 0.10512 is the *measure* of the prevalence of defective units in the lot and also indicates the probability of detecting one in a randomly drawn sample. This is no longer an *estimate* of the probability of a positive result. Instead, it is the *true probability* or *prevalence* or *population probability.* The population probability determines the estimated probabilities that we may expect from a given number of sample units examined (i.e., the observed number of sample units that test 'positive'). If the proportion of non-conforming units, (i.e., the population probability), is low the estimated probability is not likely to be precise. We can never know the true probability unless the whole lot is sampled but this is not feasible when destructive test methods are used, such as in food microbiology, because of the test time needed and because there would be no food left to eat! But it is useful to have this concept in mind. When more units are included in the sample of the population, the estimated probability is likely to be closer to the true prevalence of defective or non-conforming units.

6.3 Population and Sample of the Population

The preceding section introduced the concept of a population being all the elements or units in a lot or batch, and that the sample of the population should have attributes that are representative of the population. In terms of standard plate counts, for example, these concepts would be represented by:

(i) all the counts that would be observed by examining every unit in the lot and
(ii) the counts actually observed based on the limited number of units examined. Statisticians use the word "sample" for a *group of units* that is withdrawn to estimate the characteristics of the whole population, while an analyst or bacteriologist would refer to any one of these units as a "sample". To minimise confusion, here, the sample of the population, will refer to the whole group of units withdrawn, and the *sample units* of which the sample is composed. The assumption is also made that a sample unit is an identifiable unit that can be repeatedly recognized (e.g., a uniform-sized beefburger or unit of packaging, or the contents of a defined sampling implement). In turn, the *analytical unit* may then be a portion of the sample unit and its size is defined (e.g., in g or ml). In this chapter, however, the sample unit and the analytical unit are described as though they are the same.

6.4 Choosing the Sample Units

Sections 6.8 and 6.9 describe how to choose the material to be tested from the total amount in the lot or shipment and Chap. 9 describes the practical aspects of collecting and handling samples. The important point is to avoid bias, so that the sample will be as representative as possible of the lot. Random selection is one way of achieving this. Thus, if we think of the lot as made up of a population

of 10 g blocks which we regard as sample units, and we decide that 10 such units constitute a representative sample, then we should choose these units in such a way that each sample unit in the lot has the same chance of being included among the sample units chosen. Another approach is to use stratified random sampling (*see* Sect. 6.9) in which the lot is considered as a series of sub-lots that share some common characteristic (e.g., time of processing during a continuous processing operation; individual units from one production batch that are heat-treated in different retorts, or in the same retort but at different times). These sub-lots are then randomly sampled and tested. In practice it is often difficult to ensure random sampling, and this can be particularly significant for products with incomplete mixing or of unknown origin. At the very least, however, we should try to draw test material from all parts of the lot, whether time within a process or position in a load or vessel. Proper selection of samples is essential if the sampling plan (*see* Sect. 6.5 below) is to give unbiased results and to lead to unbiased decisions based on the decision-making criteria in the sampling plan. By sampling randomly we are able to reduce the risk of making biased decisions. In this book all statistical calculations assume that the sample has been drawn randomly, unless otherwise stated.

6.5 The Sampling Plan

The results of tests applied to the sample units will be compared with certain criteria to reach a decision as to whether the entire lot should be accepted or rejected (*see* Sect. 6.7 below for explanation of rejection). The particular choice of sampling procedure and the decision criteria is called the *sampling plan.*

A simple hypothetical example of a sampling plan follows. Take ten analytical units of 25 g of a food from a lot and assess the presence or absence of the target microorganism in each of those sample units. For this example, if two or fewer of the ten sample units show the presence of the organism (i.e., give a "positive" result), then the whole lot of food is acceptable (in relation to this organism). But if three or more sample units give a positive result, the whole lot is to be rejected. In addition to defining the attribute being assessed and the tolerable level (e.g., in this case the attribute being assessed is the presence of the organism in the 25-g samples), a sampling plan is described by two terms, namely:

n = number of sample units to be tested and
c = the maximum number of sample units that can produce a positive result for the lot still to be considered acceptable,

Also, for presence/absence testing, sample unit size is important for interpretation of the results. In the above example, the sampling plan is described as an "$n = 10$, $c = 2$" 2-class sampling plan with 25-g analytical unit size.

6.6 The Operating Characteristic Function

In Sect. 6.5, we described an $n = 10$, $c = 2$ sampling plan. If we are going to use this plan, we want to know what assurance it will give us that the plan would identify an unacceptable batch. In other words, how *discriminating* is the $n = 10$, $c = 2$ sampling plan? It is possible that, due to random effects in sampling, the plan will sometimes accept a poor lot if, by chance, we fail to sample positive units. It is also possible for the plan to reject a good lot if, by chance, we happen to draw a higher proportion of positive units in the sample than is present in the lot as a whole. There is no way to avoid some degree of error, i.e., drawing a set of sample units that, by chance, are not perfectly representative of the lot, unless we test the entire lot. We can reduce the likelihood of such random sampling errors by

testing more sample units (larger n). In fact, we can reduce the risks of incorrect lot assessment to any desired level by making n sufficiently large. In practice, however, we usually have to seek a compromise between (a) large n (many sample units) and less chance of incorrectly classifying the batch, and (b) small n (few sample units) and larger chance of incorrectly classifying the batch as acceptable when, in fact, it is not, or rejecting a batch that is actually acceptable. The first error is described as the 'consumer risk', because the consequence is that consumers are exposed to a risk above that which is considered acceptable, while the latter error is described as 'producer risk' because the producer will be penalized despite that the product is of acceptable quality or safety. This is further discussed in Sect. 6.6.3. In the language of statistics the producers risk is a Type 1 error or a 'false positive', while the consumer's risk is a Type 2 error or a 'false negative'.

6.6.1 The OC-Curve

An *operating characteristic* function is used to describe the performance of a sampling plan. This is often depicted as an operating characteristic (OC) curve (Fig. 6.1). The horizontal axis shows a measure of lot quality. One common measure of lot quality is the true proportion (or prevalence) of units in a lot that are defective, i.e., that do not conform to the criterion of acceptability (i.e., contain the target organism or have a count above some number m). This proportion is often designated p and can have values from 0 to 1 (or 0–100%). It should be emphasised that these are defectives in the sample unit, which generally will be different from the 'serving size' or 'product unit size'. The actual serving size or product unit sizes are of more relevance for the estimation of risks to public health. Another measure of lot quality would be the mean concentration or mean log concentration in the lot (see Chap. 7).

The vertical scale of the OC plot gives the probability of acceptance, P_a for a given sampling plan characterised by n and c and, for a given true prevalence of defective units/samples P_a is the *expected* proportion of occasions that the results of testing according to the sampling plan will indicate that the lot is acceptable. In other words, P_a is an indication of the reliability of the sampling plan, or of our

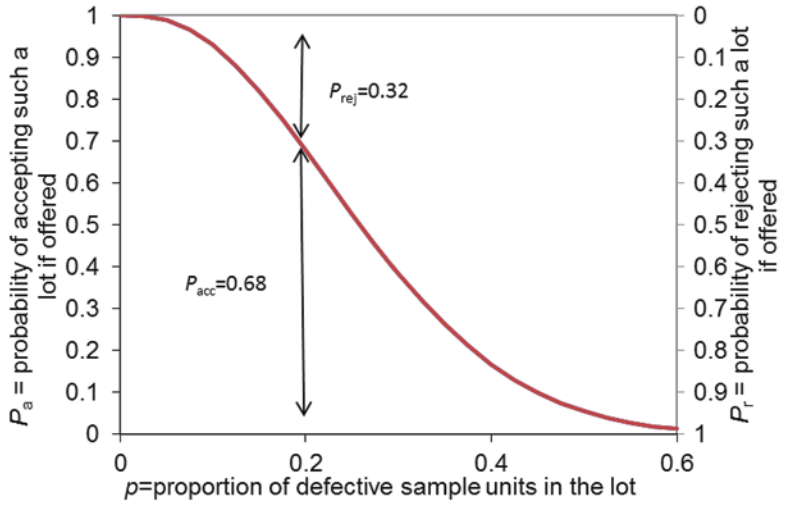

Fig. 6.1 The operating characteristic curve for a sampling plan with $n = 10$, $c = 2$, i.e., the probability of accepting lots in relation to the proportion defective among the sample units comprised in the lot being examined. If, for example, the lot comprises 20% ($p = 0.2$) defective sample units, the lot will be accepted with a probability of 0.68 and rejected with a probability of 0.32

Table 6.1 Effect of true defective rate on the probability of lot acceptance using a 2 class attributes sampling plan

p (%) (true proportion of defective units)	P_a (probability of accepting the batch using an $n = 10$, $c = 2$ sampling plan)
0	1.00
10	0.93
20	0.68
30	0.38
40	0.17
50	0.05
60	0.01

confidence that the sampling plan will not accept a lot that contains more than the proportion of defective units specified as being acceptable.

The probability of detecting an unacceptable batch (and, conversely, the probability of accepting it) is governed by the true prevalence of defective units. This is illustrated, using an *n = 10, c = 2* sampling plan, in Table 6.1.

P_a is determined using the 'Binomial Distribution'. The principal underlying the Binomial Distribution and, hence, the probability of acceptance are described in Sect. 6.6.2.

6.6.2 The Binomial Distribution

In probability theory and statistics, the binomial distribution describes the chance of finding a specific number of positives when drawing samples from a lot and applying a test that has only two possible outcomes, e.g., a positive or a negative. In microbiological testing for pathogens, in particular, this is frequently the case because it is hoped that the contaminant will be present only at low levels, if at all, and either is present in a sample unit or is not. However, a test that assesses whether a microbiological count is above or below some numerical limit *m* also gives either a positive or negative result. P_a can equally be determined using the binomial distribution.

To illustrate, consider, for example, 1000 chocolate bars of which 100 are contaminated with *Salmonella*. It might be expected that if one sample unit (bar) was chosen at random there would be a 1-in-10 chance that the sample unit contains a *Salmonella* cell. Conversely, there is a 90% chance that the contaminant would not be present in the sample unit. If two sample units are randomly drawn, each has a 1/10th chance of containing a *Salmonella* cell: the probability of *not* detecting *Salmonella* is 90% x 90%, i.e., 81%.[1] If three sample units are taken and a positive in any sample unit causes rejection, the probability of *not* detecting the contamination among three sample units is 73%, and so on. Since not detecting a *Salmonella* cell will lead to acceptance of the batch of chocolate bars, we can calculate the probability of acceptance as the product of the probability of not detecting a *Salmonella* cell in the total number of sample units tested, which can be expressed mathematically as:

[1] In fact, because sample units are not returned to the lot after sampling, the probability of detection in any subsequent sample is slightly altered after each sample is taken. This is because the population size is slightly reduced after sampling. The probability of detecting a defective sample in this situation is better described by the Hypergeometric distribution. In practical situations, however, the difference in probability of acceptance due to this consideration is insignificant (since the amount of sample taken is negligible in comparison to the total lot) and calculations based on the Binomial distributions are adequate and lead to simpler calculations.

$$P_a = (1-p_1)\times(1-p_2)\times\ldots\times(1-p_n) \tag{6.1}$$

where $p_{1,2,\ldots,n}$ is the true proportion of defective units in the lot (i.e., chance of finding a non-conforming sample unit) when 1, 2 …. n (respectively) sample units are tested. Since, in most practical situations[1], $p_1 \cong p_2 \cong \ldots p_n$ we can summarise the above equation as:

$$P_a = (1-p)^n \tag{6.2}$$

where p is the true (or 'just acceptable') proportion of defective units in the batch and n is the number of sample units from the batch that are tested. Then P_a is the probability that the sampling plan would not detect a batch with greater than the acceptable frequency of defective units.

For sampling plans in which $c > 0$ somewhat more complex calculations are needed (Eq. 6.3) but all derive from the same principles of probability.

$$P_a = \frac{n!}{c!\times(n-c)!} \times p^c \times (1-p)^{n-c} \tag{6.3}$$

Equation 6.3 is called the Binomial Distribution and Eq. 6.2 is a special case of the Binomial Distribution for which $c = 0$. To use Eq. 6.3 to calculate the probability of acceptance, the cumulative form of the function:

$$P_{a,\Sigma} = \sum_{i=0}^{c} \frac{n!}{c!\times(n-c)!} \times p^c \times (1-p)^{n-c} \tag{6.4}$$

is used.

Computer spreadsheet software often includes built-in functions to automate these calculations. For example, in Table 6.1, for the entry $p = 20\%$, $P_a = 0.68$ and can be calculated using Eq. 6.4 or can be calculated in Microsoft® Excel using the function Binomdist(2, 10, 0.2, 1) in which the first number (i.e., 2) is the number of non-conforming units that can be tolerated for the batch to remain acceptable (the c value), the second number (i.e., 10) is the number of sample units to be tested (the n value), the third number (i.e., 0.2) is the true proportion of defective units in the batch (the p value) and '1' is part of the syntax used in Microsoft® Excel to generate a cumulative probability (like in Eq. 6.4), rather than a probability density curve (like in Eq. 6.3).

Figure 6.1 shows the full operating characteristic curve for the $n = 10$, $c = 2$ sampling plan and highlights the probability of acceptance or rejection of a lot that has 20% defective units. For lots with fewer defective units the probability of acceptance using this plan is higher while for lots with a higher proportion of defective units the probability of acceptance is lower.

While we have described p here as the true proportion of defectives, to establish a sampling plan we now consider p not as the true proportion of defective units, but the maximum *tolerable* proportion of defective sample units. Adopting this approach, and by reference to the OC curve, the reliability of a given sampling plan can be evaluated. The greater the proportion of units in a lot that are defective, p, (i.e., that contain a pathogen or contain a level of microorganism above some specified level), the lower is the probability of acceptance (P_a) of that lot.

If, for example, we set an upper limit of 20% defectives (i.e., $p = 20\%$), then using the $n = 10$, $c = 2$ sampling plan, the P_a would be 0.68 for a lot that has exactly that 20% defective units. This means that on 68 of every 100 occasions when we sample a lot containing 20% defectives, we may expect to have 2 or fewer of the 10 tests showing the presence of the organism, and thus calling for acceptance, while on 32 of every 100 occasions there will be 3 or more positives, leading to the lot being considered unacceptable. As noted, an $n = 10$, $c = 2$ sampling plan has a 68% probability of correctly identifying

a lot that has 20% or more defective units. If the true rate of defectives were 30% the probability of acceptance would decline to 38% (see Table 6.1). Similarly, if p = 10% such lots will be accepted 93 of 100 times, while if p = 40%, such lots will only be accepted 17 of 100 times. Thus, using an n = 10, c = 2 sampling plan, lots with 10% defective sample units will be accepted most of the time, but with 40% defective units, acceptance would be seldom. Note that in none of these situations does the n = 10, c = 2 sampling plan guarantee a correct result in all cases.

We might consider that a sampling plan that only provides 68% confidence of rejection of a lot with >20% defective units is not stringent enough. We can also use Eq. 6.2 to identify an alternative sampling plan (with c = 0) to determine how many samples would need to be tested to be confident, at some required level, that batches with greater than some specified proportion of defective units would be rejected, as follows:

$$P_a = (1-p)^n$$

taking the logarithm of both sides:

$$\log(P_a) = n \ \log(1-p)$$

and rearranging again:

$$n = \log(P_a) / \log(1-p)$$

By substituting in the required confidence of acceptance of a non-compliant batch (which is 1 − probability of rejection of a non-compliant batch, i.e., 0.05 if we want to be 95% confident of rejecting a non-compliant batch), and the threshold of acceptability (which in this example is 20%), we find:

$$\begin{aligned} n &= \log(0.05) / \log(1-0.2) \\ &= -1.301 / \log(0.8) \\ &= -1.301 / -0.09691 \\ &= 13.43 \end{aligned}$$

i.e. 14 samples, none of which are positive, would be required to be 95% certain that the lot contains less than 20% defective units. Eq. 6.3 can equally be used for any required value of P_a and tolerable prevalence, p, to determine the number of samples required. (Using a sampling plan with c = 2, would require 30 samples to be taken to be 95% confident that the batch did not exceed 20% defectives). With both of these schemes, however, the producer's risk is increased as shown in Fig. 6.2 by the n = 14, c = 0 plan, while requiring fewer samples, results in greater producer's risk.

6.6.3 Consumer Risk and Producer Risk

As discussed in the previous section, since decisions to accept or reject lots are made on samples drawn from the lots, occasions will arise when the sample results do not reflect the true condition of the lot. As discussed above *producer's risk* describes the probability that an acceptable lot will be incorrectly rejected. *Consumer's risk* describes the probability that an unacceptable lot when tested will be inappropriately accepted, i.e., the probability of accepting a lot whose actual microbial quality is substandard as specified in the sampling plan, even though the determined values indicate acceptable quality. The consumer's risk is expressed by the probability of acceptance (P_a) of the batch given

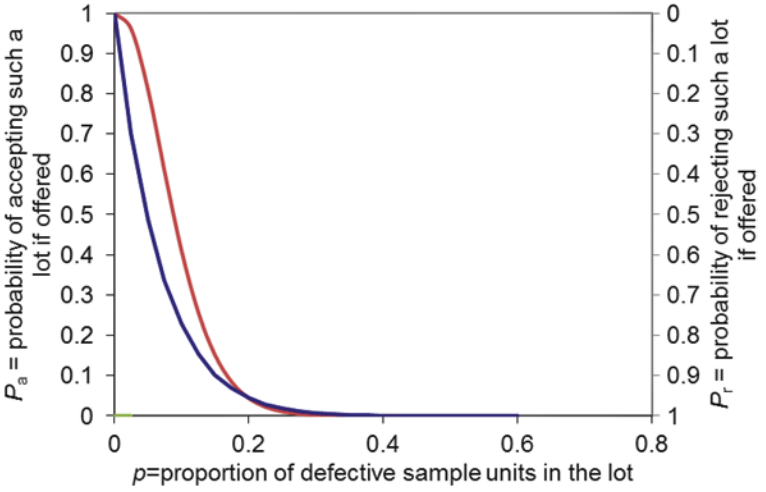

Fig. 6.2 Comparison of $c = 2$, $n = 30$ (blue line) and $c = 0$, $n = 14$ (red line) sampling schemes designed to reject batches with >20% defective units with 95% confidence. The $n = 14$, $c = 0$ plan results in increased producer's risk, but involves fewer samples

its true level of contamination, i.e., the lower the value of P_a the less likely it is that consumers will be exposed to substandard products. Conversely, the producer's risk for any level of contamination and sampling plan is expressed by the probability of rejection, $P_r = 1 - P_a$. The consumer's risk (P_a) and producer's risk ($P_r = 1 - P_a$) are depicted in Fig. 6.1.

6.6.4 Stringency and Discrimination

When sampling plans are compared and their reliability or "stringency" in making decisions is considered, different aspects of their performance can be addressed. Figure 6.3 illustrates an idealized OC curve for a sampling plan that provides perfect *discrimination* between acceptable and unacceptable lots because acceptance probabilities drop from 100% to 0 at the chosen limit of acceptability. Any lot in which the proportion of defective units exceeds the threshold for tolerance (20% in this example) will be detected with absolute certainty, and rejected.

No practical sampling plan can achieve perfect discrimination between acceptable and unacceptable lots because it would require all samples in the lot to be tested to identify an unacceptable lot. The steeper the curve, however, the closer the plan approaches that condition. Generally, steeper curves can only be achieved by increasing the number of sample units (n) to be drawn from a lot. This is illustrated in Fig. 6.4a, b which show the effect of sampling on consumer's and producer's risks as a function of the number of sample units tested. In the figure the limit for acceptance is 20% defective units within the lot. It can be seen that the probability of acceptance of a lot that has greater than the acceptable frequency of defective units (i.e., the consumer's risk) declines more rapidly when n is larger. Similarly, the probability of rejection of an acceptable batch (i.e., the producer's risk) also declines more rapidly when n is larger.

Considering the example in Sect. 6.6.2, if $n = 100$ and $c = 20$ (i.e., still tolerating 20% defective units), a batch with 15% defective units will be accepted 93% of the time while a batch with 25% defective units will be rejected 15% of the time while a batch with 40% defective units will be rejected nearly every time ($P_a < 0.00002$). In summary decisions have to be made balancing the number of

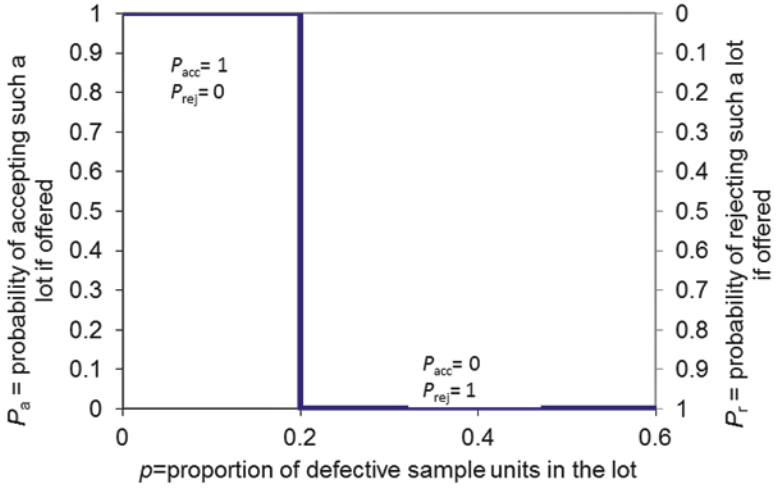
P_{acc}= 1
P_{rej}= 0
P_{acc}= 0
P_{rej}= 1
P_a = probability of accepting such a lot if offered
P_r = probability of rejecting such a lot if offered
p=proportion of defective sample units in the lot

Fig. 6.3 The operating characteristic (OC) curve for the idealized situation of complete discrimination between lots with a proportion of defective sample units below 20% and such lots with such a proportion above 20%

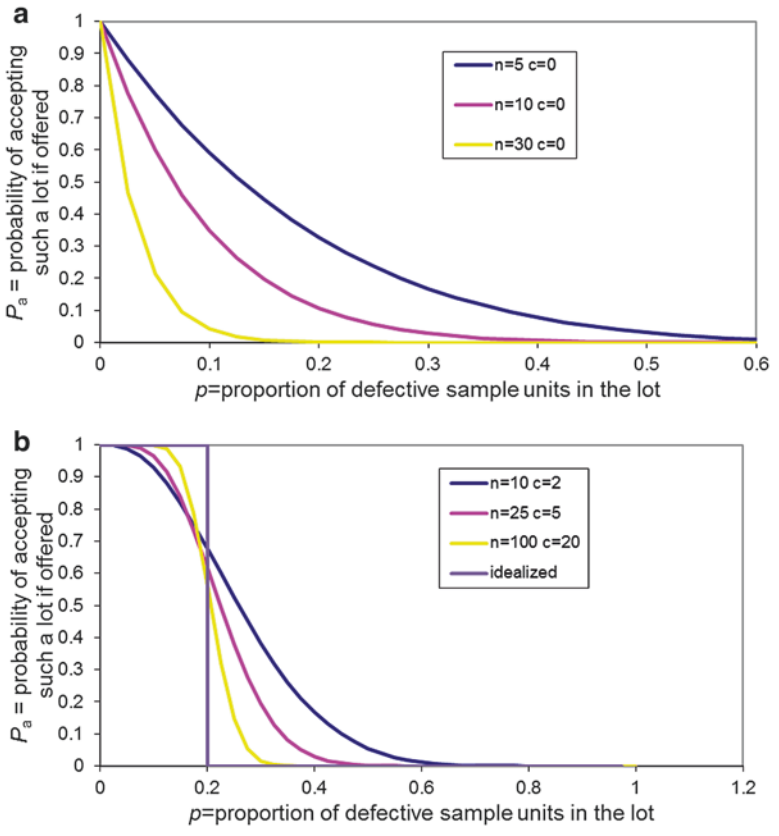

Fig. 6.4 Operating characteristic curves a) using various sampling units $n = 5$, $n = 10$, and $n = 30$ and b) having various combinations of n and c with c equal to 20% of n ($n = 10$, $c = 2$; $n = 25$, $c = 5$; $n = 100$, $c = 20$)

samples (making the OC curve more steep/moving to lower levels of defective units) and the c value (a higher value makes the curve move to the right). Together, these elements determine the steepness, location and shape of the OC curve.

6.7 Acceptance and Rejection

Acceptance or rejection of a lot, based on a sampling plan associated with some particular microbiological test or attribute, have been discussed. The judgement of acceptability or not applies only to the purpose for which that test (or several such tests) was performed. A food unsuitable for one purpose may still be suitable for another; for example, if rejected for humans, it might still be suitable for animals. Alternatively, if rejected, a food might be reprocessed, thereby decreasing the actual defect rate to a level that passes the test and becomes acceptable for the original purpose. Normally, therefore, a rejected lot will simply be withheld while the responsible food safety manager decides what to do with it, for example, to return it to the supplier, order reprocessing, forbid its use for human consumption, or order its destruction, according to circumstances. Throughout this text, the terms *accept* and *reject* are used in this limited sense, i.e., accepted or rejected for the purpose for which the sampling plan is designed.

6.8 What Is a Lot?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions (CAC 2004). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice (*see* Chap. 7). In most instances, the distribution of microorganisms within lots of food is heterogeneous and it has been shown that the logarithms of the counts from a batch of food are likely, under certain circumstances, to be normally distributed (Jarvis 2008). This heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as when it is part of an even larger quantity of the product.

It is therefore helpful if suppliers give identifiable code numbers to batches (lots) of food produced over short time periods (e.g. a day or part of a day), for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e. the producer's risk) can have severe consequences, as the whole lot will be affected by this decision, even though only few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor-quality food and, at the expense of more analyses, can result in the rejection of fewer units from the whole consignment. This is a variation on the concept of a stratified sampling plan described more fully in Sect. 6.9.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

6.9 What Is a Representative Sample?

A *representative sample* reflects, as far as is possible, the composition of the lot from which it is drawn.

How then should a representative sample be drawn? It is important to avoid bias and to draw a sufficient number of sample units to confidently make a judgement about a lot. Sampling at random is the universally recognized way of avoiding bias. The units (cartons or containers, particular weights

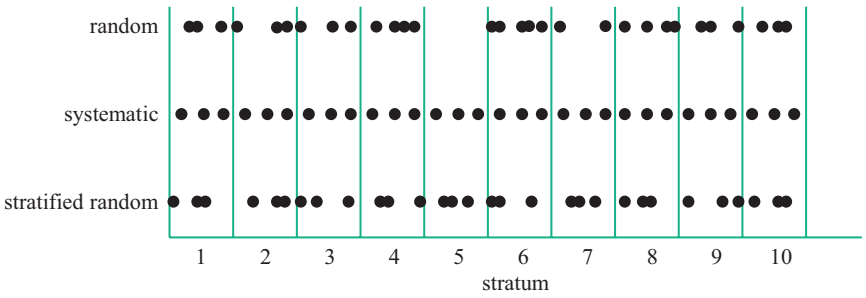

Fig. 6.5 Illustration of random, systematic and stratified random sampling of 30 sample units distributed over 10 strata. With a random distribution the 30 points are randomly distributed over all 10 strata, resulting also in a probability that in a certain stratum no samples are taken. For systematic sampling all 30 samples are taken equidistantly in time or place. For the random stratified sampling exactly an equal number of samples is taken in every stratum, in this case three. But the moment the samples are taken in the stratum is random (Adapted from Jongenburger (2012))

of solid, or volumes of liquid) for testing are selected by using random numbers. There is, of course, no guarantee that a random sample has characteristics identical with those of the lot, but the randomness of sampling is the basis for calculation of the probability that a sample will give a certain result and results in a better chance of encountering all the variation in the lot.

It is also possible, and maybe desirable, to use a *stratified random sampling* approach, i.e. drawing at random a given number of sample units from each *stratum* (e.g. each sub-lot or carton). The proportion of sample units from each stratum should correspond to the proportion of lot units in that stratum. This means, that if each stratum contains the same quantity of product as every other stratum the numbers of sample units per stratum should be the same, otherwise they should differ according to the proportion of the lot contained in the various strata that are being assessed. Stratification is a method for handling known sources of variation, and may be used where one has prior knowledge that the consignment is potentially not of uniform quality. A consignment might not be of uniform quality if portions of it are shipped in different vehicles or in different holds of a vessel, or if it is known that it is really composed of several lots, representing, for example, different days of production from the same plant, different plants of the same company, or from different suppliers. The results for different strata should be assessed separately and then pooled if they appear to be homogeneous. Jongenburger et al. (2011a, b) have shown that the performance of totally random, systematic and stratified random sampling (Fig. 6.5) are equal for a homogenous contamination but that, in certain circumstances, systematic or stratified random sampling is more effective if there are 'hot spots' (Jongenburger et al. 2011b; Kiermeier et al. 2011) of contamination. Considering that random sampling performs less well with a clustered contamination and that systematic sampling might perform less well with systematic contamination (e.g., dripping of condensation, sequential fillers), stratified random sampling can be considered the method of choice.

Where physical or practical constraints prevent random sampling the calculated probabilities may not be valid. Their reliability is dependent on how closely reality approaches the ideal situation of random sampling. This should be borne in mind when subsequently interpreting results.

6.10 Confidence in Interpretation of Results

As discussed above, test results based on sampling plans are not able to give total assurance that the decision will be correct. There are many factors, which can affect our confidence in the sampling results obtained. Many of these are related to the methodologies we use. In this chapter, it is assumed that the analytical procedure is certain to detect a contaminant if present, and that it will give no false

positives. In practice, these assumptions are never correct and, even for standard microbiological methods, details of the performance of the analytical procedure, like sensitivity and specificity or repeatability and reproducibility, may not be available. Knowledge of such information sometimes could lead to a modified calculation of the OC function or to different critical limits (*see* Chap. 10).

The way in which the sample is handled and manipulated can also have an effect on our ability to identify accurately the type or quantity of contaminant(s) present. The choice of handling procedure will vary according to the material under examination, the preservation system used and the method of analysis. The choice of such handling procedures requires expert microbiological judgement.

Statistical considerations are related to our sampling plan assumptions as outlined previously. Particular attention should be given to the sampling plan OC curve (stringency and discrimination) and to the method of sampling, since the calculation of acceptance probabilities is only reliable if sample units are taken at random. With regard to quantitative analytical results, the design of sampling plans relies on knowledge about underlying distributions and the typical variability between sample units. This is reflected either by the calculation of acceptance probabilities or by the choice of critical limits. These issues are discussed in greater detail in Chap. 7. In addition, it is important to consider whether the whole sampling protocol and assumptions implied are suitable for the intended or desired decision to be made about the lot.

Finally, it is important to remember that many non-statistical considerations may influence the choice of a sampling scheme. For example, the economic cost of sampling and the risk of cross-contamination due to excessive sampling (opening multiple containers). The need for rapid results with perishable or commercially sensitive foods can require adoption of methods that are not statistically ideal or sampling plans with little discrimination (Powell 2013). There are many sampling plans utilized in food microbiology, e.g., $n = 5$ or even $n = 1$, that are mainly motivated by such issues. These low numbers represent self-imposed, non-statistical constraints that limit the confidence one can have in the correctness of decisions based on those sampling plans.

To be fit for the intended purpose, a sampling plan should be designed with regard to these constraints and the validity of assumptions made. The choice of the sampling plan, including critical limits, and the number of sample units to be analysed, should be chosen according to the intended purpose. The choice of n, in particular, should reflect the stringency appropriate to the decision being made about the lot, e.g., whether a product supports the growth of a dangerous pathogen, or whether the test is applied as an indicator of hygiene or general microbiological quality. The recommended procedure would be to firstly set desired acceptance and rejection probabilities for lots of defined acceptable and unacceptable qualities, and then to derive the number of sample units required to reliably discriminate such lots.

6.11 Practical Considerations

To use available resources to the best effect, not all food can receive the same attention. Foods representing the highest risk, whether to public health or for exacting buyer specifications, should receive the most time and effort, which means that they should be subjected to the most intensive sampling. What factors govern this decision? The following is a list of some important factors for consideration.

(a) **Hazard.** The most important factor is the hazard involved. How hazardous is the type (or types) of microorganisms present? And how hazardous are the numbers likely to be present? These questions are discussed more fully in Chap. 8. The probability of incorrectly accepting a lot should be reduced with increasing risk.

(b) **Uniformity.** If the food has relatively few loci of contamination, even if the number of contaminants is very high, then the chance of detection is low. On the other hand, if the same food is thoroughly mixed, and the contaminants are more uniformly spread, they will appear in more sample units. The chance of detection is then higher with any given sampling plan.
(c) **Stratification.** If it is known that there is stratification within lots of the food (*see* Sect. 6.9), a corresponding stratification can be used in selecting sampling units.
(d) **Record of consistency.** A consistently good record for a food from a specific source indicates that its control is reliable, and may justify reduced sampling or even omission of sampling of occasional lots. Discretion must be used in making this decision, with increasing confidence as the record accumulates. Confidence will be maintained if the producer provides detailed records of control procedures. Skip-lot sampling is a statistical procedure that can be used under such circumstances and is discussed in Chap. 11. Chapter 13 discusses methods for assessing the overall statistical control and reliability of processes.
(e) **Practical limitations.** Since regulatory agencies rarely have the resources to test all imported lots, they will often have to reduce the number of samples in sampling plans to a feasible level to be able to test more lots. Most microbiological tests are laborious and slow; and regulatory agencies may not be able to hold highly perishable foods pending results of analyses. Political or administrative pressures to reduce sampling may increase the probability of error.

In considering the above factors, it must be remembered that the ability to distinguish between acceptable and unacceptable lots often improves relatively little compared with the increase in the number of sample units withdrawn from the population. Indeed, over most of the realistic range of P_a, reliability only increases roughly as the square root of the number of sample units, so that multiplying the number of sample units four-fold will only approximately halve the likelihood of making wrong decisions.

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Chapter 7
Sampling Plans

7.1 Introduction

This text is concerned primarily with plans that may be applied to lots of food presented for acceptance at ports or other points of entry. Often, little or no information is available to the receiving agency about the method by which the food was processed or the record of previous performance by the same processor. Under these circumstances, attributes plans are appropriate. Variables sampling plans (see Sect. 7.3), which depend upon the nature of the frequency distribution of microorganisms within lots of foods, are suitable only if this distribution is known. Furthermore, variables sampling plans are not suited for presence/absence testing. This limits severely their usefulness in port-of-entry sampling, but they may be particularly helpful to food producers monitoring their own production.

7.2 Attributes Plans

7.2.1 Two-Class Attributes Plans

A simple way to decide whether to accept or reject a food lot may be based on a microbiological test performed on a certain number of sample units (n). This will usually be a test for the presence (positive result) or absence (negative result) of a microorganism. Concentrations of microorganisms can be assigned to a particular attribute class by determining whether they are above (positive) or below (negative) some preset concentration.

As explained in Chap. 6, the decision-making process is defined by two numbers. The first is represented by the letter n, and defines the number of sample units required for testing. The second number, denoted c, is the maximum allowable number of sample units yielding unsatisfactory test results, for example, the presence of the organism, or a count above the defined concentration, denoted m, which in a two-class plan separates good from defective units (Fig. 7.1a). Thus, in a presence/absence decision on a lot, the sampling plan $n = 10$, $c = 2$ means that ten sample units are taken and tested; if two or fewer show the presence of the organism, the lot is accepted (with respect to this characteristic); but if three or more of the ten show the presence of the organism, the lot is rejected, although not necessarily destroyed (see Chap. 6, Sect. 6.7, for an explanation of rejection).

Only under circumstances where the lot is intimately mixed can data obtained from the application of a two-class plan based on presence/absence of a microorganism be used to give an approximate

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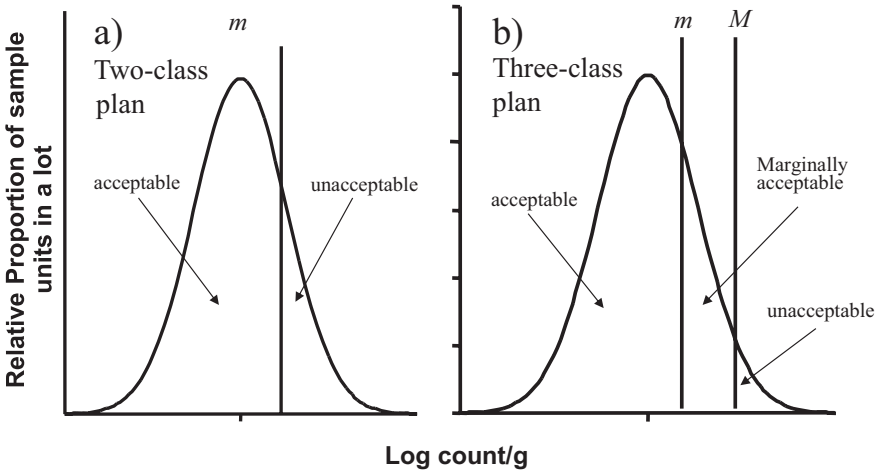

Fig. 7.1 Two and three-class attributes plans

guide on the likely concentration of that microbe within the lot. Such techniques should only be applied when the analyst is confident that there is a uniform distribution of the microorganisms of concern.

The performance of the sampling plan depends upon n and c (see Chap. 6, Sect. 6.6.4). The larger the value of n at a given value of c, the better is the distinction between acceptable and unacceptable lots. Thus, compared with $n = 10$, $c = 2$, the plan $n = 15$, $c = 2$ is more stringent, while the plan $n = 5$, $c = 2$ is more lenient. On the other hand, for a given sample size n, if c is decreased, the better the food must be to have the same chance of being passed. Conversely, if c is increased, the plan becomes more lenient and will more often pass food lots with unacceptable quality,[1] as the probability of acceptance P_a increases. Probabilities of acceptance for a set of plans are given in Tables 7.1 and 7.2. Figure 7.2 displays the operating characteristic (OC) curves for a few of these plans to illustrate the characteristics of various two-class attributes plans.

The importance of the sample size, n, is also stressed when we take a different approach to assess lot quality. Suppose a sampling plan was applied not only to decide between acceptance and rejection of the lot, but also to give an estimate of the proportion of defectives in the lot. Such estimates should be reported in terms of confidence intervals, giving a range of values and supported by the actual sampling results. The width of these ranges, i.e., the precision of the estimates, is greatly influenced by n. For illustration purposes, lower und upper limits of 95% confidence intervals are given in Table 7.3 for some combinations of sample sizes, n, and actual numbers of positive sample units, k, for a homogenous contamination having a certain defective rate per sample.

Another aspect that might be considered is the influence of the size of the lot. A sample size of, say, $n = 30$ sample units might be taken at random from a lot of any size (or consignment, if appropriate – see Chap. 6, Sect. 6.8 and Chap. 17, Sect. 17.5.3.2) for use in a two-class plan with $c = 0$. If the lot contains a very large number of sample units, one obtains an OC curve such as that shown in Fig. 7.2c. According to that OC curve, a lot with one defective unit out of 40 (proportion defective, $p = 0.025$) will be accepted about half the time ($P_a = 0.47$) when using the plan $n = 30$, $c = 0$. Calculation of this probability is based on the binomial distribution model.[2]

[1] The use of the word quality in this chapter includes safety. It can relate for example to total counts, indicator organisms or specific pathogens, depending on the microbiological criterion at stake.

[2] If the sampling process is without replacement (which generally will be the case), the probabilities of acceptance P_a

Table 7.1 Two-class plans ($c = 0$): probabilities of acceptance (P_a) of lots containing indicated proportions of acceptable and defective sample units

Composition of lot		Number of sample units tested from the population (n)							
% acceptable ($100 - p$)	% defective (p)	3	5	10	15	20	30	60	100
99	1	0.97	0.95	0.90	0.86	0.82	0.74	0.55	0.37
98	2	0.94	0.90	0.82	0.74	0.67	0.55	0.30	0.13
97.5	2.5	0.93	0.88	0.78	0.86	0.60	0.47	0.22	0.080
95	5	0.86	0.77	0.60	0.46	0.36	0.21	0.046	0.006
90	10	0.73	0.59	0.35	0.21	0.12	0.042	<	<
80	20	0.51	0.33	0.11	0.035	0.011	<		
70	30	0.34	0.17	0.028	<	<			
60	40	0.22	0.078	0.006					
50	50	0.13	0.031	<					
40	60	0.064	0.010						
30	70	0.027	<						
20	80	0.008							
10	90	<							

'<' means $P_a < 0.005$.

In summary, a sampling plan gives nearly the same degree of protection against acceptance of unacceptable lots as against rejection of acceptable ones, regardless of the size of the lot. The attributes schemes given in this book do not increase stringency as lot size increases. The sampling plan is, therefore, independent of the size of the lot, provided the lot is large in comparison to sample size (as always will be the case). If the important criterion is the actual number of defective units, rather than the proportion of the lot that is defective, then the sampling plans given here are not appropriate.

7.2.2 Three-Class Attributes Plans

Three-class attributes plans (Bray et al. 1973) were devised for situations where the quality of the product can be divided into three attribute classes, depending upon the concentration of microorganisms within the sample units. Counts above a concentration m, which in a three-class plan separates good from marginally acceptable units, are undesirable but some can be accepted. However, a count above a second concentration M for any sample unit is unacceptable, and if any count for the n sample units from a lot exceeds M that lot is rejected (Fig. 7.1b). This concept is based on the idea that analytical results for sample units drawn from a lot are of a quantitative nature. In this case, quantities of microorganisms in sample units can be described in terms of frequency distributions that can be characterized by some measures of location and spread.

Figure 7.3 illustrates the effect of the values of m and M for three-class plans for various frequency distributions of microbial content within a lot. Curve 1 represents an entirely satisfactory lot, with low numbers of bacteria generally, and thus a low average count with little variation and no counts exceeding m. Curve 2 represents a lot with a similar average count, but with a much wider variation, so that

for various values of p should, in principle, be calculated using a different distribution model (hypergeometric). This effect only becomes important when a quarter to a half of the lot is taken as a sample, a circumstance that realistically never occurs in bacteriological analysis of lots of food.

Table 7.2 Two-class plans (selected c values): probabilities of acceptance (P_a) of lots containing indicated proportions of acceptable and defective sample units

Composition of lot		$n = 5$			$n = 10$			$n = 15$			$n = 20$		
% acceptable $(100 - p)$	% defective (p)	$c = 3$	$c = 2$	$c = 1$	$c = 3$	$c = 2$	$c = 1$	$c = 4$	$c = 2$	$c = 1$	$c = 9$	$c = 4$	$c = 1$
99	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	0.98
98	2	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00	0.96	1.00	1.00	0.94
97.5	2.5	1.00	1.00	0.99	1.00	1.00	0.98	1.00	1.00	0.95	1.00	1.00	0.91
95	5	1.00	1.00	0.98	1.00	0.99	0.91	1.00	0.96	0.83	1.00	1.00	0.74
90	10	1.00	0.99	0.92	0.99	0.93	0.74	0.99	0.82	0.55	1.00	0.96	0.39
80	20	0.99	0.94	0.74	0.88	0.68	0.38	0.84	0.40	0.17	1.00	0.63	0.069
70	30	0.97	0.84	0.53	0.65	0.38	0.15	0.52	0.13	0.035	0.95	0.24	0.008
60	40	0.91	0.68	0.34	0.38	0.17	0.046	0.22	0.027	0.005	0.76	0.051	<
50	50	0.81	0.50	0.19	0.17	0.055	0.011	0.059	<	<	0.41	0.006	<
40	60	0.66	0.32	0.087	0.055	0.012	<	0.009			0.13	<	
30	70	0.47	0.16	0.031	0.011	<		<			0.017		
20	80	0.26	0.058	0.007	<						<		
10	90	0.081	0.009	<									
5	95	0.023	<										

n number of sample units tested from the population; '<' means $P_a < 0.005$

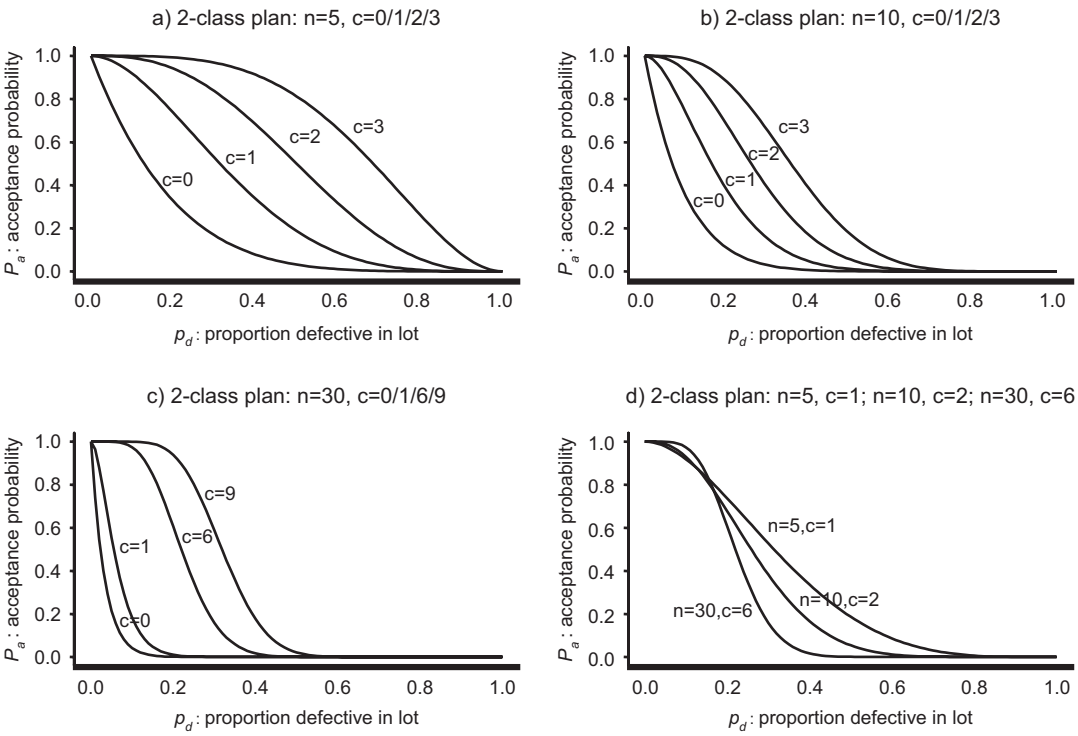

Fig. 7.2 Operating characteristic curves for different sample sizes (n) and differenct criteria of acceptance (c) for 2-class attributes plans

Table 7.3 Lower and upper limits of 95%-confidence intervals for the estimated proportion defective based on k positive results when n sample units are analysed

n	k	Lower limit	Upper limit
5	0	0.000	0.451
5	1	0.005	0.716
5	2	0.053	0.853
5	3	0.147	0.947
10	0	0.000	0.259
10	1	0.003	0.445
10	2	0.025	0.556
10	3	0.067	0.652
15	0	0.000	0.181
15	1	0.002	0.319
20	0	0.000	0.139
20	1	0.001	0.249

a small proportion of sample units would have counts exceeding m, though none exceed M. If the proportion in the range m to M were small, the situation would be acceptable; if this proportion were larger, it might still be acceptable, but it would serve as a warning call to the producer, as tending toward the situation shown in curve 3. Curve 3 represents a lot with a higher average count and larger variation, such that a small proportion of sample units exceeds M and would result in immediate rejection, while a substantial proportion falls in the range m to M, which itself could also suffice to justify rejection (see Sect. 6.7, Chap. 6 for explanation of the term *rejection)*. Curve 4 represents a lot of even greater unacceptability, requiring rejection.

Hence the definition of a three-class sampling plan incorporates two limits, m and M, M being higher than m, which distinguish three classes of sampling results. Furthermore, the number of sample

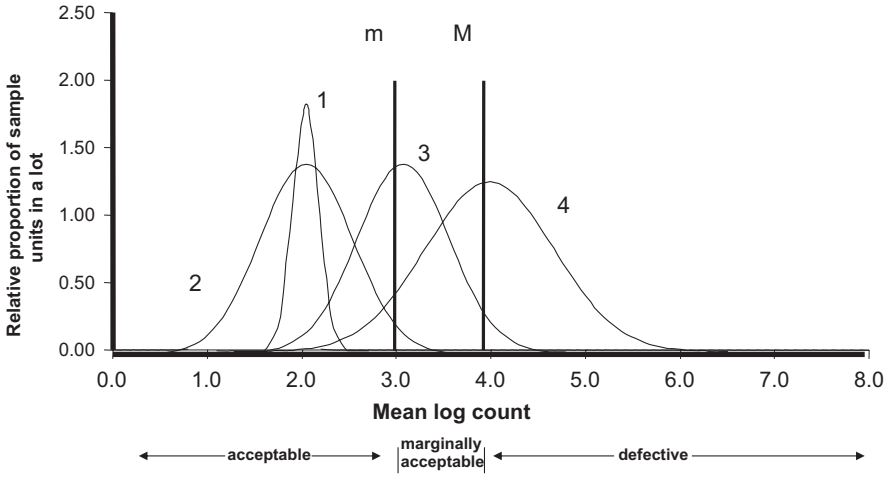

Fig. 7.3 Three class attributes plans. *m* count above a defined concentration, separating good quality from marginally acceptable quality, *M* count above a second defined concentration, separating marginally quality from unacceptable quality

units to be drawn from the lot *n*, and the maximum number of sample units *c* that are allowed to fall into the region between *m* and *M* need to be defined. The maximum number that may exceed *M* is almost always set to 0, as it is in the plans in this book.

Accordingly, in the three-class plans there are again only two numbers, *n* and *c*, from which it is possible to find the probability of acceptance, P_a, for a food lot of given microbiological quality. To describe the lot quality, we consider all sample units that could be drawn from the lot, which must yield counts in three classes: below *m*, between *m* and *M*, and above *M*. Since the proportions in the lot for the three classes must total 1, one need only specify two of them in describing lot quality. We might call these proportions the proportion *defective*, i.e., above *M* (p_d), and the proportion *marginally acceptable,* i.e., from *m* to *M* (p_m). The proportion *acceptable*, equal to or less than *m*, must be 100% minus the sum of p_d and p_m. By appropriate calculations, we can find the probability of acceptance, P_a, for a given lot quality for any specified sampling plan. For example, for the plan $n = 10$, $c = 2$, P_a will be 0.21 for a lot distribution for which 20% of the sample counts are marginally acceptable ($p_m = 20\%$) and 10% defective ($p_d = 10\%$). That is, on the basis of the particular values decided upon for *m* and *M*, only about 21 lots out of 100 of that quality will be accepted, because they have no 'defective' counts and two or fewer 'marginally acceptable' counts out of the ten sample units chosen from the lot. The other lots will all be rejected.

Probabilities associated with a collection of three-class plans are shown in Table 7.4 for various lot qualities. For acceptance or rejection, the scheme depends not only on the proportion of defective material (p_d) but also on the proportion of marginally acceptable product (p_m). The example given above (20% of marginal units and 10% defective units, $n = 10$, $c = 2$, accepted 21% of the times) can be found in this table. Using the identical plan $n = 10$, $c = 2$ a lot containing 0% defective units but 40% of marginal units has a lower chance of acceptance (17%).

Table 7.4 gives only some examples of acceptance probabilities for selected combinations of proportions p_m and p_d. To gain an impression of the overall behaviour of a three-class attributes plan, the complete operating characteristic (OC) function should be referred to. Compared with two-class plans, the OC functions of three-class plans are more complex and more difficult to visualize as their values depend on combinations of two proportions, p_m and p_d, and not only on one. Because of these dependencies and the variety of lot qualities that can occur, for a three-class sampling plan, the OC

Table 7.4 Three-class plans: probabilities of acceptance (P_a) of lots containing indicated proportions for selected numbers of sample units and c values

p_m									
p_d	10	20	30	40	50	60	70	80	90
$n = 5$, $c = 3$									
50	0.03	0.03	0.02	0.01	<				
40	0.08	0.07	0.06	0.04	0.02	<			
30	0.17	0.16	0.15	0.12	0.07	0.03	<		
20	0.33	0.32	0.31	0.27	0.20	0.12	0.04	<	
10	0.59	0.58	0.56	0.52	0.43	0.32	0.18	0.06	<
5	0.77	0.77	0.75	0.69	0.60	0.47	0.31	0.14	0.02
0	1.00	0.99	0.97	0.91	0.81	0.66	0.47	0.26	0.08
$n = 5$, $c = 2$									
50	0.03	0.02	0.01	<					
40	0.08	0.06	0.04	0.02	<				
30	0.16	0.14	0.11	0.06	0.02	<			
20	0.32	0.29	0.24	0.16	0.09	0.03	0.01	<	
10	0.58	0.55	0.47	0.36	0.23	0.12	0.05	0.01	<
5	0.77	0.72	0.63	0.50	0.35	0.20	0.09	0.02	<
0	0.99	0.94	0.84	0.68	0.50	0.32	0.16	0.06	0.01
$n = 5$, $c = 1$									
50	0.02	0.01							
40	0.06	0.04	0.01	<					
30	0.14	0.09	0.05	0.02	<				
20	0.29	0.21	0.13	0.06	0.02	0.01	<		
10	0.53	0.41	0.27	0.16	0.07	0.03	0.01	<	
5	0.70	0.55	0.38	0.23	0.12	0.05	0.01	<	
0	0.92	0.74	0.53	0.34	0.19	0.09	0.03	0.01	
$n = 10$, $c = 3$									
40	0.01								
30	0.03	0.02	0.01	<					
20	0.10	0.08	0.05	0.02	<				
10	0.34	0.29	0.20	0.10	0.03	0.01	<		
5	0.59	0.51	0.36	0.20	0.08	0.02	<		
0	0.99	0.88	0.65	0.38	0.17	0.05	0.01	<	
$n = 10$, $c = 2$									
30	0.02	0.01	<						
20	0.09	0.06	0.02	0.01	<				
10	0.32	0.21	0.10	0.04	0.01	<			
5	0.55	0.39	0.20	0.08	0.02	<			
0	0.93	0.68	0.38	0.17	0.05	0.01	<		
$n = 10$, $c = 1$									
30	0.02	<							
20	0.07	0.03	0.01	<					
10	0.24	0.11	0.04	0.01	<				
5	0.43	0.21	0.08	0.02	<				
0	0.74	0.38	0.15	0.05	0.01	<			

Each of these blocks of numbers, relating P_a to p_m and p_d, represents a three-dimensional relation called an OC surface, corresponding to the two-dimensional OC curve

'<'means $P_a < 0.005$

p_d percent defective, p_m percent marginal

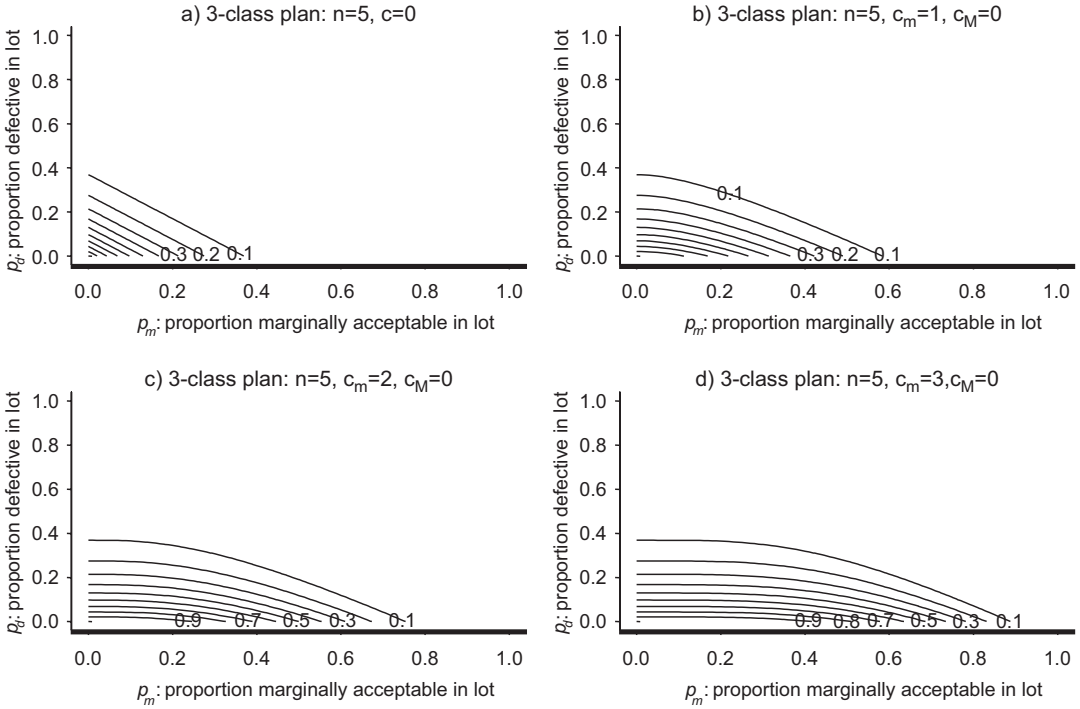

Fig. 7.4 Contour maps of operating characteristic function of 3-class attributes plan for sample size n = 5 and different criteria of acceptance(c). Numbers within graphs (e.g. 0.1, 0.2, 0.3 in graph a) represent probablity of acceptance

function should be plotted as an OC surface, either in a three-dimensional graph, or as a contour map in the two-dimensional (p_m, p_d) area with contour lines for selected acceptance probabilities. Such OC function maps are shown in Fig. 7.4 for three-class sampling plans with $n = 5$ and different acceptance numbers $c = 0$, $c = 1$, $c = 2$, and $c = 3$.

All lots with combinations of p_m and p_d lying on the same contour line in such a graph have the same probability of acceptance that is indicated at the end of the line. If, for instance, the three-class plan $n = 5$, $c = 1$ is applied, all kinds of lots with (p_m, p_d) combinations on the outermost line are accepted with a probability of only 0.1 or 10% of the times such a lot will be examined. Thus, the three-class attributes scheme is affected to some extent by the frequency distribution of microorganisms within the batch, but the advantages of the scheme are its simplicity and general applicability, which make it appropriate to port-of-entry sampling.

However, there is a need to elaborate sound methods to set the values of m and M. These should be related to actual concentrations of microorganisms and the frequency distributions of analytical results. There are statistically-based techniques for achieving this, although assumptions must be made. An example, based upon assumptions that can readily be checked and found (historically) reasonable, is Dahms and Hildebrandt (1998), which is explained in more detail in Sect. 7.4.4.

7.3 Variables Plans

Where the underlying distribution of microorganisms within the lot is known, or can be assumed, there is the option to use variables sampling plans. When properly applied, such plans can prove more useful under some conditions than the attributes plans for a particular purpose. Variables plans make full use of the microbial counts, rather than ascribing the counts to categories or ranges.

7.3.1 Identification

To define a variables plan, the underlying frequency distribution for the microorganisms of concern within the sample units in a lot must be known. There are many types of frequency distributions, and they differ in complexity. An overview of aspects of distributions for microorganisms in foods is described by ILSI (Basset et al. 2010). Some of the simpler distributions are symmetrical in shape and can be described by their mean and some measure of the distribution about that mean. The normal, or Gaussian, distribution is one such example. The normal distribution is defined by its mean value (which is also the median) for the range of concentrations found, and a measure (σ, the standard deviation), which defines the possibility of finding any other concentration in a unit. More complex distributions may not be symmetrical, so that the mean is unlikely to equal the median.

What is essential to apply a variables plan is knowledge of frequency distribution. To achieve this, data may be required to support the application of a particular distribution. The more complex the frequency distribution (i.e., more parameters are needed to define it), the more data are needed to gain confidence that it is an appropriate distribution. An example of a distribution often observed for microorganisms in foods, the log-normal distribution, is used below (Sect. 7.3.3). It is worth noting that the scale has been adjusted to obtain a normal distribution. This adjustment results in a symmetrical, two parameter (mean and standard deviation) distribution, achieved by using log (concentration) instead of concentration.

It is important to remember that any measurement of the parameters for a distribution is based upon a sample and is therefore an estimate of those parameters. These measured parameters must accommodate the uncertainty implicit in the measurement. The smaller the sample size n, the larger the likely error could be. The example given in Sect. 7.3.3 illustrates this principle by allowing for sample size in its decision matrix.

7.3.2 Prescribing Confidence in Decisions

Making critical microbiological decisions about the safety or quality of a lot of food involves three steps. The first is to define the acceptable limits for the lot, the second is to specify the confidence with which we wish to identify acceptable and unacceptable lots, and the third is to choose the appropriate sampling plan. The following is an example of the way in which a variables plan may be designed. In this case, the decision rule is based upon an assumption that the underlying distribution of contaminants in the lot is log-normal (i.e., the log of the concentrations is normally distributed). While this assumption is often correct, in practice, its justification needs to be clear and recorded. Assuming a log-normal distribution, sampling plans based on the characteristics of this distribution can be used to develop acceptance sampling plans.

7.3.3 Operation

It is necessary to obtain and handle samples and sample units in the same way as for attributes plans. The log-transformation of the concentration measurements is used to compute the sample mean ($\overline{x}$) and standard deviation (s). These two values are then used to make the decision whether to accept or reject the lot. The lot is rejected if $\overline{x} + k_1 s > V$, where V is a log-concentration related to safety/quality limits.

The value k_1 is obtained by reference to appropriate tables and is chosen to define the stringency of the plan for a given number of sample units, n.

Table 7.5 k_1 values calculated using the non-central *t*-distribution – safety/quality specification (reject if $\bar{x} + k_1 s > V$)

		Number of sample units *n*							
Probability (*P*) of rejection	Proportion (p_d) exceeding *V*	3	4	5	6	7	8	9	10
0.95	0.05	7.7	5.1	4.2	3.7	3.4	3.2	3.0	2.9
	0.1	6.2	4.2	3.4	3.0	2.8	2.6	2.4	2.4
	0.3	3.3	2.3	1.9	1.6	1.5	1.4	1.3	1.3
0.90	0.1	4.3	3.2	2.7	2.5	2.3	2.2	2.1	2.1
	0.25	2.6	2.0	1.7	1.5	1.4	1.4	1.3	1.3

Values from Malcolm (1984)
$\bar{x}$ sample mean, *V* log-concentration related to safety/quality limits

Table 7.6 An example of aerobic plate counts for a sample of poultry ($n = 5$)

APC	$\log_{10}$(APC)	Mean log ($\bar{x}$)	Standard deviation (*s*)
40,000	4.602		
69,000	4.839		
81,000	4.909	5.039	0.378
200,000	5.301		
350,000	5.544		

Selection of k_1 Table 7.5 contains a range of k_1 values for sample unit numbers between 3 and 10 (Malcolm 1984). To choose k_1 it is necessary to decide on the maximum proportion p_d of the units in the lot that can be accepted with a concentration above the limit value, *V*. Having selected p_d, the desired probability *P* can be chosen, where *P* is the probability of rejecting a lot which contains at least a proportion p_d above *V*.

For example, if five sample units are analysed per lot, then the k_1 value can be chosen from Table 7.5. If a lot in which 10% of sample units exceeded *V* is to be rejected with a probability of 0.95, then the k_1 value 3.4 would be used.

In practice, the two values p_d and *P* will be selected along with the value *V*. The scheme then allows *n* to be selected over the range 3 to 10. The larger *n* becomes, the lower the chance of rejection of an acceptable lot.

Selection of the limit value V The limit value *V* is selected by the microbiologist as the safety or quality limit, expressed as log-concentration. This value is likely to be numerically very similar to the value *M* used in the three-class attributes plans (Sect. 7.2.2).

Table 7.6 gives the results for the aerobic plate count (APC) analyses of five sample units obtained from a lot of poultry. An appropriate variables sampling plan might be $P = 0.90$, $p_d = 0.25$, with a limit value of $V = 7$. The k_1 value, obtained from Table 7.6, is 1.7. Applying the formula $\bar{x} + k_1 s$, gives 5.039 + 1.7 x 0.378, which equals 5.682. This value is less than the limit value of 7, and the lot is therefore accepted.

The use of variables plan for good manufacturing practice Food producers often find it advantageous to specify a Good Manufacturing Practice (GMP) standard. It may be possible to apply the variables plan under these circumstances, applying the formula outlined previously. The criterion is to accept the lot if $\bar{x} + k_2 s < V$. The k_2 value for the GMP plan is obtained from Table 7.7. The values *P* and p_d are selected as before and the appropriate k_2 value is obtained. The limit value, *V*, will be very similar numerically to the limit value, *m*, used in the three-class attribute plan.

For a more extensive treatment of the variables plan topic see Kilsby (1982), Kilsby et al. (1979), Malcolm (1984) and FAO/WHO (2016). The first three references describe the approach as described here without assuming a specific standard deviation, while in FAO/WHO (2016) the approach is explained for a given assumed standard deviation.

Table 7.7 k_2 values calculated using the non-central t-distribution – GMP limit (accept if $\bar{x} + k_2 s < v$)

			Number of sample units n						
Probability (P) of acceptance	Proportion (p) exceeding V	3	4	5	6	7	8	9	10
0.90	0.05	0.84	0.92	0.98	1.03	1.07	1.10	1.12	1.15
	0.10	0.53	0.62	0.68	0.72	0.75	0.78	0.81	0.83
	0.20	0.11	0.21	0.27	0.32	0.35	0.38	0.41	0.43
	0.30	0.26[a]	0.13[a]	0.05[a]	0.01	0.04	0.07	0.10	0.12
	0.40	0.65[a]	0.46[a]	0.36[a]	0.30[a]	0.25[a]	0.21[a]	0.17[a]	0.16[a]
	0.50	1.09[a]	0.82[a]	0.69[a]	0.60[a]	0.54[a]	0.50[a]	0.47[a]	0.44[a]
0.75	0.01	1.87	1.90	1.92	1.94	1.96	1.98	2.00	2.01
	0.05	1.25	1.28	1.31	1.33	1.34	1.36	1.37	1.38
	0.10	0.91	0.94	0.97	0.99	1.01	1.02	1.03	1.04
	0.25	0.31	0.35	0.38	0.41	0.42	0.44	0.45	0.46
	0.50	0.47[a]	0.38[a]	0.33[a]	0.30[a]	0.27[a]	0.25[a]	0.24[a]	0.22[a]

Values from Malcolm (1984)
$\bar{x}$ sample mean, v log-concentration limit value
[a]Negative values

7.4 Comparison of Sampling Plans

7.4.1 General Remarks

The decision for, or the design of, a suitable sampling plan depends on the given purpose, i.e., on the sampling material, the type of microbiological result being assessed, and on available prior information on production processes and frequency distributions of sampling results in lots. In the following paragraphs some statistical aspects of the choice of a sampling plan will be discussed comparing two-class with three-class attributes plans and three-class attributes plans with variables plans.

Only when the result of a microbiological analysis is given as a count, or in another quantitative manner, is there a choice between types of sampling plans. For mere qualitative results (presence-absence tests) only two-class plans are applicable.

When dealing with quantitative analytical results for sample units in a lot, questions arise concerning the frequency distributions of sample results and whether there is any previous information on shape, location and spread of these distributions. Is a typical distribution / shape expected to occur? Is the production process known and well documented? Especially for the design of variables sampling plans, some knowledge and data concerning the production processes and variations in distributions that may occur between lots is required. Because variables plans are based on the assumption that log-transformed sampling results follow a normal distribution, they should only be used when this assumption can be justified. For such situations the performance of attributes and variables plans can be compared.

The following considerations are restricted to this special situation. Sampling plans will be compared by means of their OC function calculated and plotted for various scenarios. As lot distributions are assumed to be of the 'normal' type (after log-transformation), lot quality is described by the log mean concentration of microbes for all units comprised by the lot μ, and the standard deviation σ as a measure of variation. Therefore acceptance probabilities are calculated for lots with varying μ and σ. With σ held fixed the OC curve of a sampling plan can be plotted as a function of varying mean concentrations μ for all three types of sampling plans.

7.4.2 Determining the Concentration of Microorganisms Controlled by Attributes Sampling Plans

As outlined in Chap. 3, to compare the equivalence of different control measures it is necessary to be able to relate their performance in terms of achieving an FSO. In other words, the performance of each control measure needs to be expressed as the resultant frequency or concentration of a microbiological hazard in a food. This is essential if the use of microbiological criteria is to be validated as a control measure to achieve a given FSO through controlling the initial level of a hazard (see Chap. 3, Sect. 3.5.1, Example 4 and Zwietering et al. 2015).

The method for relating the performance of attributes plans to concentration is to use the frequency distribution of analytical results in sample units to establish the proportion of defective samples, as proposed by Hildebrandt et al. (1995). A normal distribution for the log concentration of microbes is assumed, and the area under the normal density function below m is used to define the value for the proportion acceptable. The area between m and M defines the value for proportion marginally acceptable (p_m), and the area above M (or m for a two-class plan) defines the value for proportion defective (p_d). The mathematics involved in calculating the three proportions for a given mean log concentration are detailed in Legan et al. (2000). For calculations with presence-absence tests, details can be found in van Schothorst et al. (2009).

OC curves expressed in terms of mean concentration are developed by fixing the standard deviation σ, and then increasing the mean of a normal distribution through a range of values. Figure 7.5 illustrates this for a two-class plan with $n = 5$, $c = 0$, $m = 1.0$ log cfu/g. A distribution with $\sigma = 0.8$ and three different means is shown in Fig. 7.5a**.** The σ value of 0.8 is chosen based on published concentrations of mesophilic *Clostridium* spores in raw pork, beef and chicken (Greenberg et al. 1966) and similar observations in other food materials. All parts of the distribution above m in each position are defective. The proportion defective in the distribution in each position (or in a position defined by any other mean) is plotted against mean log count to show how the proportion defective increases with mean log count (Fig. 7.5b). Finally the operating characteristic curve for the specified plan is used to determine the probability of acceptance from the proportion defective at each mean log count, for a plan with five samples taken. This probability of acceptance is plotted against mean concentration (Fig. 7.5c).

The protection each sampling plan gives can then be expressed in terms of the mean concentration of microorganisms associated with a defined probability of accepting the sampled material. In Chap. 8 this approach will be used to compare the concentration of microorganisms controlled under different cases.

7.4.3 Comparison of Two-Class and Three-Class Attributes Plans

Table 7.8 compares the operating characteristics of the two types of attributes plans recommended in this text, on the basis of equal sample sizes n, acceptance numbers c, and lot qualities. To facilitate comparison, lot quality is measured as the proportion of the lot worse than level m and, correspondingly, the same value of c is taken for the two-class plan as the c of marginally acceptable units for the three-class plan.

The two-class plans do not distinguish values between m and M from those above M, as do three-class plans. If not more than c sample units give results above m, the lot is acceptable, regardless of how far individual results exceed m. But the corresponding three-class plan does make a distinction, by including an extra subdivision of lot quality since the limit M separates marginally acceptable from defective units.

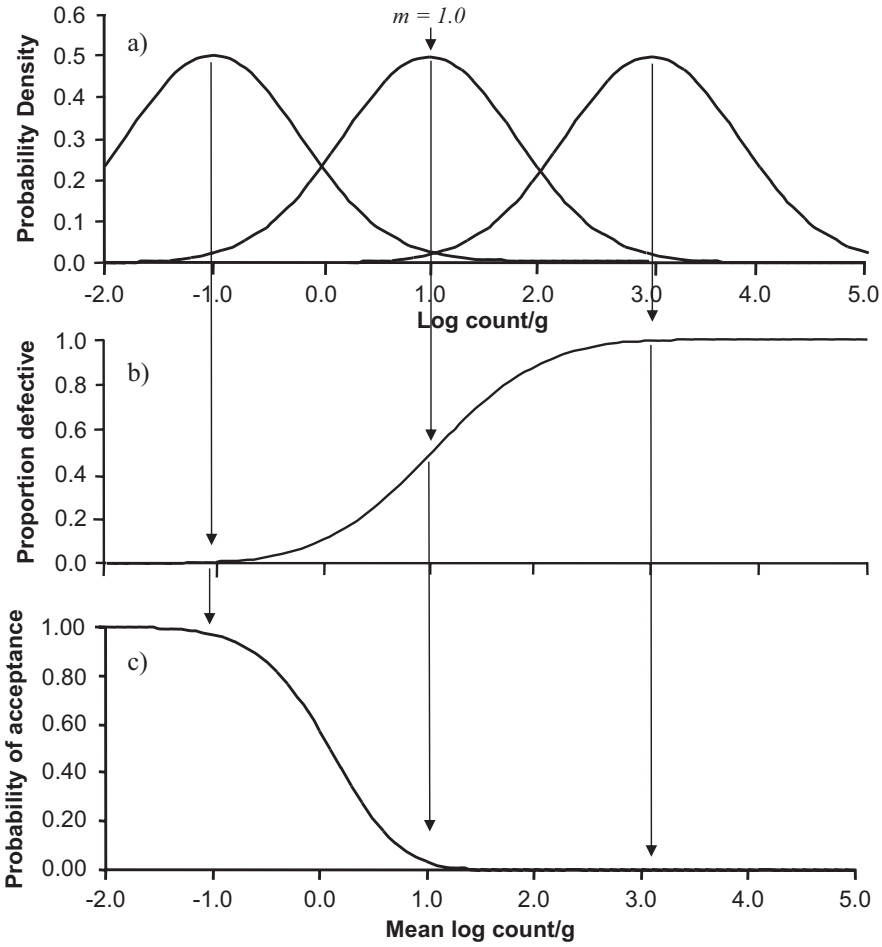

Fig. 7.5 OC curves expressed in terms of mean concentration. (**a**) is the probability density of three distributions with three different mean concentrations. (**b**) is the proportion defective as function of the mean concentration, (**c**) is the probability of acceptance with a sampling plan with $n = 5$ and $c = 0$

By comparing OC surfaces for three-class plans with a fixed number of sample units n, but with varying values for c (Fig. 7.4) it becomes obvious that the surface heights change mainly in the p_m-direction, i.e., for varying proportions of marginally acceptable units in the lot. The reason is that the number of sample units that are allowed to exceed M remains constant at 0. In fact a three-class plan might be interpreted as a mixture of two two-class plans, of a two-class plan (n, c) referring to the limit m, and of the two-class plan (n, 0) referring to the limit M. In extreme situations one of these two-class plans can be dominating the decision process. Generally however, the actual performance of a three-class plan depends on the variety of combinations of p_m and p_d that are likely to occur in practise.

Hildebrandt et al. (1995) studied the performance aspects of two-class and three-class sampling plans in case log-normal lot distributions can be assumed by comparing the two-class plan $n = 5$, $c = 1$; $m = 5 \times 10^4$ CFU/ml with the three-class plan $n = 5$, $c = 1$; $m = 5 \times 10^4$, $M = 10^5$ CFU/ml.

Four different types of lots characterized by lot standard deviations of $\sigma = 0.1$, $\sigma = 0.2$, $\sigma = 0.4$, and $\sigma = 0.8$ were considered to study the impact of the standard deviation of the lot in relation to the

Table 7.8 Probabilities of acceptance (P_a) for two- and three-class attributes sampling plans when the lot examined contains 5%, 20%, or 50% of sample units > m

		5% > m			20% > m				50% > m			
Sampling plan		Two-class plan	Three-class plan		Two-class plan	Three-class plan			Two-class plan	Three-class plan		
n	c		0% > M	5% > M		0% > M	10% > M	20% > M		0% > M	25% > M	50% > M
5	0	0.77	0.77	0.77	0.33	0.33	0.33	0.33	0.03	0.03	0.03	0.03
5	1	0.98	0.98	0.77	0.74	0.74	0.53	0.33	0.19	0.19	0.11	0.03
5	2	1.00	1.00	0.77	0.94	0.94	0.58	0.33	0.50	0.50	0.19	0.03
5	3	1.00	1.00	0.77	0.99	0.99	0.59	0.33	0.81	0.81	0.23	0.03
10	0	0.60	0.60	0.60	0.11	0.11	0.11	0.11	<	<	<	<
10	1	0.91	0.91	0.60	0.38	0.38	0.24	0.11	0.01	0.01	0.01	<
10	2	0.99	0.99	0.60	0.68	0.68	0.32	0.11	0.05	0.05	0.02	<
10	3	1.00	1.00	0.60	0.88	0.88	0.34	0.11	0.17	0.17	0.03	<
15	0	0.46	0.46	0.46	0.04	0.04	0.04	0.04	<	<	<	<
20	0	0.36	0.36	0.36	0.01	0.01	0.01	0.01	<	<	<	<
60	0	0.05	0.05	0.05	<	<	<	<	<	<	<	<

Note that any % > M is included in the % > m; e.g., for the case where 20% > m and 10% > M, it follows that 10% is between m and M
'<' means $P_a < 0.005$

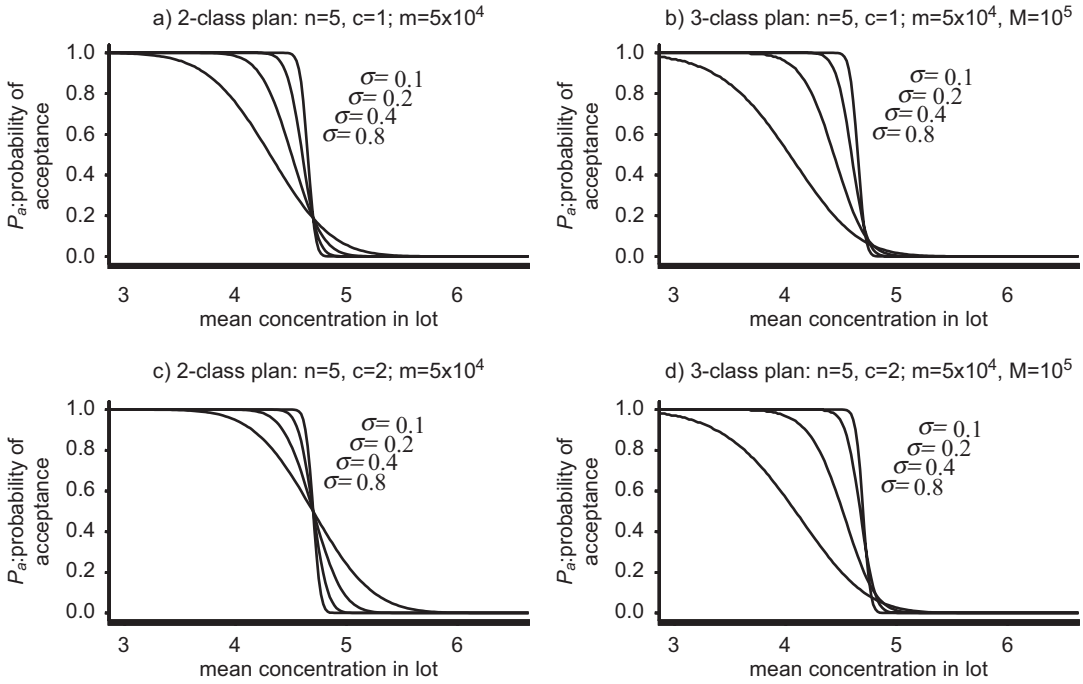
a) 2-class plan: n=5, c=1; m=5x10^4
b) 3-class plan: n=5, c=1; m=5x10^4, M=10^5
c) 2-class plan: n=5, c=2; m=5x10^4
d) 3-class plan: n=5, c=2; m=5x10^4, M=10^5
Pa:probability of acceptance
mean concentration in lot
σ= 0.1
σ= 0.2
σ= 0.4
σ= 0.8

Fig. 7.6 Comparison of 2- and 3-class attributes plan with different criteria of acceptence (c) when distribution of log-transformed analytical results is assumed to be 'normal'

distance between m and M on the operation of the three-class sampling plan. In Fig. 7.6a the OC curves for the two-class plan are shown, Fig. 7.6b contains the OC curves for the three-class plan. The OC function values are calculated by deriving the proportions of marginal and defective units, p_m and p_d, resulting from the various possible combinations of μ and σ with given microbiological limits $m = 5 \times 10^4$ and $M = 10^5$.

As can be seen for lot distributions with a low standard deviation ($\sigma = 0.1$) in relation to the distance between m and M there is hardly any difference in performance between the two-class and the three-class sampling plan. As the within-lot standard deviation is increased in relation to the distance between m and M, the OC difference becomes larger showing reduced acceptance probabilities when the three-class plan is applied.

If the number of acceptable units in the sample, c, is changed from $c = 1$ to $c = 2$ the effect on the operation of two-class plans is much more obvious than the effect on three-class plans (Fig. 7.6c, d), especially for lots with higher standard deviation. This example demonstrates the dominance of the $c_M = 0$ rule (the null-tolerance concerning M, incorporated in three-class plans when lots are examined that are characterized by high standard deviation in relation to the distance between m and M).

A plot of the possible combinations of p_m and p_d in a given sampling situation together with the contour lines of the three-class plan serves to summarize these points (Fig. 7.7). When lots are homogenous (standard deviation is low in relation to the distance between m and M; or m and M are different in relation to lot standard deviations likely to occur), the three-class plan (n, c) will operate like a simple two-class plan with (n, c) accordingly. If lots are heterogeneous (standard deviation is high in relation to the distance between m and M; or the distance from m to M is narrow in relation to lot standard deviations likely to occur), the three-class plan will operate like a two-class plan with (n, $c_M = 0$). Hence the performance of three-class plans depends not only on the combination of n and c, but on m and M and their distance in relation to lot heterogeneity.

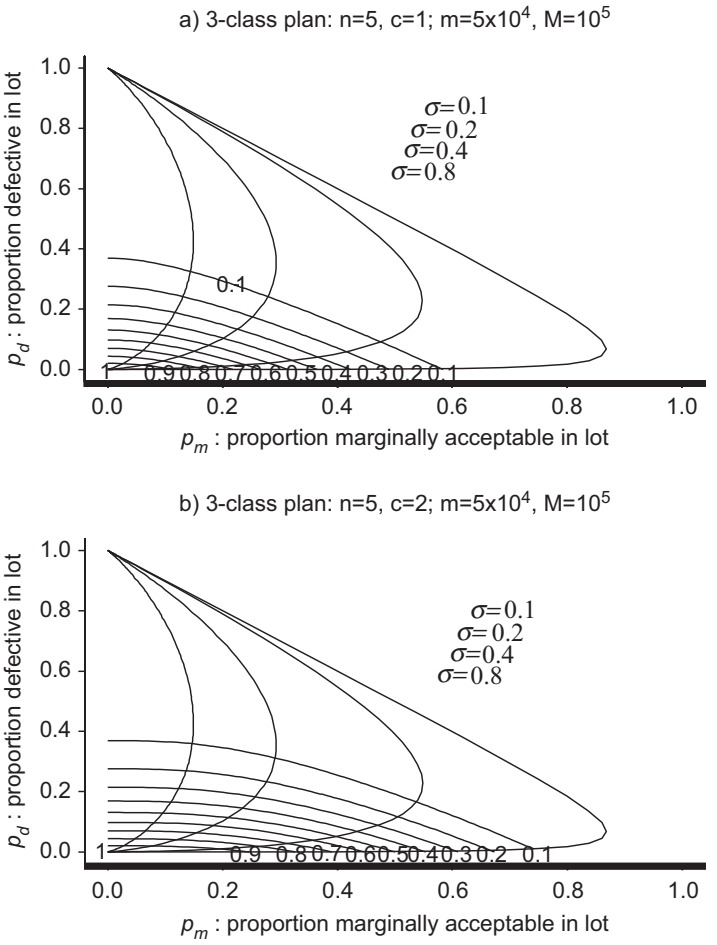
a) 3-class plan: n=5, c=1; m=5x10^4, M=10^5
σ=0.1
σ=0.2
σ=0.4
σ=0.8
0.1
1 0.9 0.8 0.7 0.6 0.5 0.4 0.3 0.2 0.1
p_d : proportion defective in lot
p_m : proportion marginally acceptable in lot
b) 3-class plan: n=5, c=2; m=5x10^4, M=10^5
σ=0.1
σ=0.2
σ=0.4
σ=0.8
1 0.9 0.8 0.7 0.6 0.5 0.4 0.3 0.2 0.1
p_d : proportion defective in lot
p_m : proportion marginally acceptable in lot

Fig. 7.7 Contour maps for 3-class attributes plan and possible combinations of proportion marginally acceptable (p_m) and defective (p_d) in lot, for various standard deviation (σ) when distribution of analytical results is assumed to be 'normal'

7.4.4 Construction of Three-Class Plans Using Previous Information

The considerations in the preceding section started with given microbiological limits *m* and *M* without questioning the reasons for their selection. Often, however, these limits are described as the maximum level of target organism *m*, acceptable under conditions of good manufacturing practice (GMP) and the limit of target organisms *M*, that, if exceeded, is considered unacceptable, i.e., defective.

This reference to GMP-conditions, and to empirical studies describing frequency distributions for quantitative analytical results that are achievable under these conditions, implies that the design of sampling plans could be based on knowledge about production technologies leading to values for *m* and *M* that take into account the maximum mean concentration of contaminants, as well as the maximum extent of heterogeneity, under conditions of good manufacturing practice.

As an example, guidance for defining the microbiological limits with regard to GMP-conditions has been given by Dahms and Hildebrandt (1998). They addressed the problem that there might be an unduly high probability of rejecting a lot still meeting GMP-conditions if the distance between *m* and *M* is too narrow in relation to the acceptable heterogeneity. Especially with regard to non-pathogenic microorganisms like total bacterial count or indicators of hygiene that represent no health risk for the consumer, it is questionable whether to reject a lot meeting GMP-requirements solely because of a single sampling result exceeding *M*. In this context, the difference (*M* - *m*) should be chosen such that lots characterized by marginal concentrations of microorganisms, and by acceptable or even unavoidable heterogeneity, run only a minor, and known, risk of rejection due to a single sample unit lying above *M*.

Based on the assumption that sampling results (after log-transformation) follow a normal distribution, an 'indifferent lot' for the two-class sampling plan $n = 5$, $c = 2$, is first considered. The term 'indifferent lot' indicates that there is an equal probability of 0.5 to accept the lot or to reject it. The definition of this two-class sampling plan implies that an indifferent lot is characterized by a mean concentration of microorganisms just at the limit m: $\mu = m$, i.e. just at the maximum acceptable mean concentration under conditions of good manufacturing practice. As soon as lot mean concentrations exceed this limit, the probability of rejecting the lot will be greater than the probability of accepting it. Therefore the hypothesis tested with this two-class plan is one concerning the mean concentration of contaminants of the lot being examined.

Application of a three-class plan to the same lot with $n = 5$, $c = 2$, i.e., the introduction of a second limit M and the requirement $c_M = 0$, can lead to a reduction of acceptance probabilities. However, whether the resulting difference between using the two-class and the three-class plans is relevant or not depends on the distance between m and M in relation to lot heterogeneity (Sect. 7.4.3). With regard to these relationships, it is proposed to define the additional risk of rejecting an indifferent lot with a given, acceptable, heterogeneity (standard deviation) as a first step, i.e., to define the required reduction of acceptance probability for a lot with marginal mean concentration of contaminants $\mu = m$ and marginal spread σ. As a second step, a value for the upper limit M should be chosen that meets this requirement.

For the situation when previous information indicates that log-transformed sampling results follow a normal distribution with a known standard deviation σ that is achievable under GMP-conditions, Dahms and Hildebrandt (1998) derived a formula to calculate the required distance between m and M:

$$M - m = u_{1-p_d^*}\sigma$$

Here $u_{1-p_d^*}$ is the $(1-p_d^*)$-quantile of the standard normal distribution, with p_d^* being the marginal acceptable proportion of defectives exceeding M. This value can be calculated as:

$$p_d^* = \frac{5}{8} - \sqrt{\frac{25}{64} - \frac{4}{5}\cdot a}$$

for $n = 5$, $c = 2$ and the additional risk to reject an indifferent lot given as a. Table 7.9 lists some values for the distance between m and M for different combinations of a and σ.

These considerations illustrate how to select M, once m has been set. This procedure is mainly oriented towards the design of sampling plans for non-pathogenic microorganisms such as indicators of hygiene. However, the relationship between M and m could be used in a similar way for pathogens. One would simply start by first setting M with regard to safety, and choosing m accordingly.

Following a procedure like this a three-class sampling plan can be constructed to meet defined requirements concerning its stringency in comparison with the equivalent two-class plan. A characteristic of the three-class plan is the statement of two hypotheses that are implicitly tested when this sampling plan is applied, one concerning the marginal mean concentration of contaminants of an acceptable lot as m, and the other concerning the marginal spread that is acceptable by fixing the distance between m and M.

Table 7.9 Distances between m and M for given additional risks a to reject indifferent lots with acceptable heterogeneity (standard deviation) σ for a three-class sampling plan with $n = 5$, $c = 2$

a	p_d^*	$u_{1-p_d^*}$	$\frac{M-m}{\sigma = 0.2}$	$\sigma = 0.4$	$\sigma = 0.8$
0.01 (1%)	0.006	2.51	0.502	1.004	2.008
0.05 (5%)	0.033	1.84	0.368	0.736	1.472
0.10 (10%)	0.068	1.49	0.298	0.596	1.192

7.4.5 Comparison of Three-Class Attributes and Variables Plans

The three-class plan is a simple plan to apply. It relies upon ascribing a concentration measurement to one of three broad concentration bands. In order to achieve this simplicity, it sacrifices discrimination. For example, if m is 1000 and M is 10,000, then the three-class attributes plan assigns the same level of concern to 1001 and 9999 contaminants in a sample unit. Also, it assigns a totally different level of concern to 999 and 1001 contaminants. The variables plan described above has the advantage that it has high discrimination between individual concentration measurements. On the other hand, the variables plan is mathematically more complex to operate and to understand, and its performance depends upon the validity of the assumptions made about the frequency distribution.

Taking the three-class plan $n = 5$, $c = 2$; $m = 5 \times 10^4$, $M = 10^5$ as an example, the operation characteristic of this attributes sampling plan can be compared with that of a variables sampling plan with $n = 5$, $V = 10^5 = M$, $p_0 = 0.05$ and $L_0 = 0.95$. Figure 7.8 shows the OC curves for the variables sampling plans when the lot standard deviation σ is assumed to be known in comparison with the OC curves for the three-class sampling plan already discussed under Sect. 7.4.3. It should be noted that

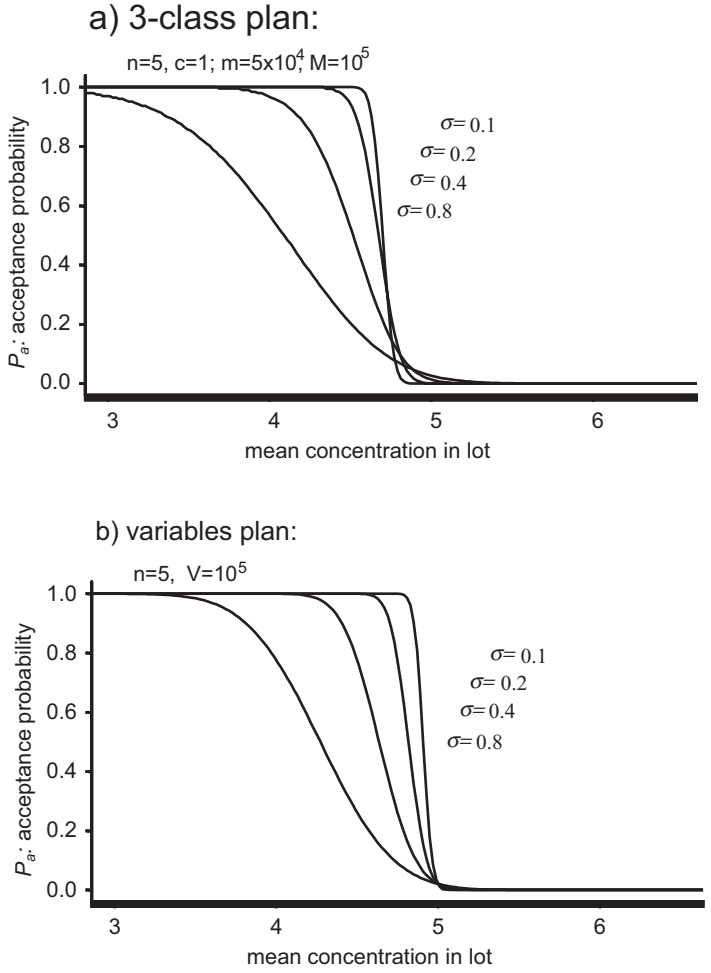

Fig. 7.8 Comparison of 3-class attributes plan and variables plan with standard deviation of concentrations in lot assumed to be known

the decision rule of the variables plan is unable to respond if the actual standard deviation is larger than the assumed σ, whereas the decision rule of the three-class plan remains the same, responding to higher measured concentrations. In this comparison we can see that, for homogenous lots, both plans have a high stringency in discriminating between lots of good and of bad quality. However, whereas the three-class plan discriminates focussing on the marginal lot mean concentration m, the variables plan discriminates at mean concentrations nearer to $V = M$, as this is the starting point to define the marginal mean contamination $V - k \times \sigma$ that is relevant for this type of plan.

With increasing lot standard deviation the slopes of both OC curves become less steep, this effect being stronger for the three-class plan. The variables plan remains more stringent, i.e., acceptance probabilities for lots of acceptable quality remain quite high, whereas they fall more rapidly than those of the three-class plan as soon as lot quality changes from acceptable to unacceptable.

Closer operation comparisons of these types of sampling plans would be achieved if previous information is used in the design of the three-class plan, especially in choosing the appropriate distance between m and M. However, it is important to realize that these two approaches are fundamentally different. Because the bases for decision making are fundamentally different, the two approaches should not be expected to be generally equivalent. The attributes schemes were designed for situations where no assumptions about underlying distributions could be made (e.g., port-of-entry), and they will perform well under those circumstances against their set parameters. When it is possible to have high confidence in underlying frequency distributions (e.g., in food manufacturing units) alternative approaches may allow for different sets of assumptions and more cost effective sampling plans related to these assumptions. Therefore, choice of sampling plan should depend upon knowledge of the lot and its intended use.

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Chapter 8
Selection of Cases and Attributes Plans

8.1 Introduction

While the overall philosophy of this book is to control microbial hazards through raw material selection, GHP and HACCP, and not to rely on microbiological testing, there are occasions when testing might be considered. If it is concluded that testing is appropriate, this chapter provides guidance on the choice of sampling plan and discusses their limitations. The recommended sampling plans are based on statistical considerations in Chaps. 6 and 7, severity of the hazard and change in risk (decrease, no change, or increase) before a food is consumed. The International Commission on Microbiological Specifications for Foods (ICMSF) recommended 15 cases that reflect different levels of risk (ICMSF 1974, 1986). The greater the risk, the higher the case number, and the more stringent the sampling plan (see Sect. 8.5 and Table 8.1).

The principles for establishing microbiological criteria were described in Chap. 5. Chapter 5, however, does not describe how to choose a sampling plan, an essential component of all microbiological criteria. Due to the importance and complexity of choosing a sampling plan, Chap. 6 discusses the concept of probability and factors to consider when collecting representative samples from a lot or consignment of food. In Chap. 7, two basic sampling plans (2-class and 3-class attributes plans) are described. The choice of sampling plan should take into account a number of factors, including the risk to public health associated with each hazard and susceptibility of the target group of consumers. This chapter incorporates information from the previous chapters and provides a scheme that can be used in deciding on a sampling plan that is based on risk.

The stringency of sampling plans for foods should be based on the hazard to the consumer from pathogenic microorganisms and their toxins or toxic metabolites, or on the potential for quality deterioration to an unacceptable state. Plans should also take account of the types of microorganisms present and their numbers. Some microorganisms merely spoil a food, others can cause illness, and still others are taken to indicate the likelihood of contamination by pathogens. Some pathogens cause mild illnesses which seldom spread; others cause mild illnesses which spread rapidly; and yet others cause severe illness. The degree of concern for a foodborne hazard is often increased if the organism has grown to high numbers in the food and, conversely, is usually reduced if the number is reduced. In some cases the food merely acts as a vehicle for transmission of the infectious microorganism. Treatment in the normal course of distribution, storage, and preparation for consumption may decrease, leave unchanged or increase numbers of microorganisms, while labile toxins would decompose and stable toxins remain.

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Table 8.1 Plan stringency (case) in relation to degree of risk and conditions of use

Type of hazard	Conditions in which food is expected to be handled and consumed after sampling, in the usual course of events		
	Reduced risk	Cause no change in risk	May increase risk
Utility (e.g. general contamination, reduced shelf life, spoilage)	Case 1	Case 2	Case 3
Indicator; low, indirect hazard	Case 4	Case 5	Case 6
Moderate hazard, not usually life threatening, usually no sequelae, normally of short duration, symptoms are self-limiting, can be severe discomfort	Case 7	Case 8	Case 9
Serious hazard, incapacitating but not usually life threatening, sequelae rare, moderate duration	Case 10	Case 11	Case 12
Severe hazard for (a) the general population or (b) restricted populations, causing life threatening or substantial chronic sequelae or illness of long duration	Case 13	Case 14	Case 15

The choice of sampling plan for microbiological criteria should first reflect the purpose. For example, is the microbiological criterion intended to assess the general quality and acceptability of a food i.e., utility, assess the microbiological safety either indirectly (i.e., an indicator organism) or directly (i.e., a pathogen, toxin, toxic metabolite), or verify that a food safety system if operating as intended (see Chap. 13).

8.2 Microbial Criteria: Utility, Indicator and Pathogenic Microorganisms

8.2.1 Utility Microorganisms

Some microbiological tests provide information regarding general contamination, incipient spoilage or reduced shelf life. Evidence should support the use of a utility test for the intended purpose. For example, evidence should support the use of a total aerobic count as measure of incipient spoilage. Such tests may be useful indicators of product quality. However, utility tests are not related to health hazards but rather to economic and aesthetic considerations, therefore the level of concern is low. Utility tests are included in cases 1–3 (see Table 8.1) and satisfied by relatively lenient sampling plans. They may involve direct microscopic counts, yeast and mold counts, aerobic plate counts, or specialized tests such as for cold-tolerant microorganisms or for species causing a particular type of spoilage, e.g., lactobacilli in mayonnaise, or thermophilic spore-formers in sugar.

8.2.2 Indicator Microorganisms

Microorganisms that are not normally harmful, but may indicate the presence of pathogenic microorganisms may be used as indirect indicators of a health hazard (see also Chap. 5 Sect. 5.7.1.1). For example, for dried egg products, *Enterobacteriaceae* can be used as an indicator of the presence of salmonellae. In these products, any practically applicable sampling plan cannot detect the low level of salmonellae, if present. It is important to recognize that relationships between pathogen and indicators are not universal and are influenced by the product and process. Care must be taken when selecting indicator microorganisms. For instance, “coliform counts” have been widely used as universal indicators of hygiene, but apparently high coliform counts do not necessarily indicate hygienic failure, since

they can be already intrinsically present in the raw materials. Microorganisms naturally present in the product may also interfere with the analysis and result in meaningless counts. For instance, aeromonads are often detected by coliform count procedures, rendering counts of coliforms meaningless for many seafood products where aeromonads are common members of the microbiota.

Indicator microorganisms may be useful in other situations, e.g., when assessing the efficiency of cleaning and disinfection or in investigational sampling. A food plant laboratory may prefer not to test for a specific pathogen (salmonellae or *L. monocytogenes*), because culturing these organisms in the laboratory could increase the risk of the microorganism being introduced into the food processing environment. Therefore, a generic test, e.g., *Enterobacteriaceae* may be considered safer.

An inspector at a port of entry may know very little about the history of a consignment of food, e.g., whether a heat process adequate to kill relevant microorganisms was used in processing, whether the food was contaminated after processing, or whether the consignment was temperature-abused during shipment. Tests for relevant microorganisms can indicate whether certain foods have been under-processed. For instance, high numbers of mesophilic spore-forming bacteria in low-acid, shelf-stable canned foods indicate probable under-processing when it is certain the container has not developed a leak. The presence of *Enterobacteriaceae* or coliforms in some properly pasteurized foods indicates re-contamination after heat processing. *E. coli* in water indicates recent fecal contamination, and *S. aureus* in cooked foods can indicate contamination from the human skin or nose. Because of the uncertain relationship between indicators and specific pathogens, the level of concern is moderate and it is inappropriate to apply sampling plans with a high stringency for indicator microorganisms.

8.2.3 Pathogens

There are occasions when testing for a pathogen may help to ensure food safety. These include:

- routine sampling when experience indicates that testing is an effective means of consumer protection;
- verification of GHP/HACCP systems when a suitable indicator microorganism is not available; and
- investigational sampling: either when the epidemiology of a foodborne disease outbreak points to a particular ‘lot’ of food as the cause of illness, or when there are other circumstances creating suspicion of the presence of a pathogen or toxic metabolite (see Chap. 11).

8.3 Factors Affecting the Risk Associated with Pathogens

Microbiological criteria and sampling plans should reflect the severity of the disease and be appropriate for the food. Certain well-known food-pathogen combinations have become recognized. Some understanding of the conditions that determine whether a food is likely to contain pathogens or their toxic metabolites is necessary. Frequently, there are strong regional and cultural influences on these associations.

8.3.1 Epidemiologic Considerations

Water and some types of seafood have been shown to be common vehicles in outbreaks of typhoid, cholera, and hepatitis A infection. Meat and poultry are commonly identified as vehicles in outbreaks of salmonellosis. Ham and cream-filled pastries are frequently implicated in outbreaks of

staphylococcal foodborne illness. Outbreaks of gastroenteritis caused by *Vibrio parahaemolyticus* are usually associated with seafood. Cooked meat and cooked poultry, or stews and gravy that have been subjected to time-temperature abuse after cooking, are the usual vehicles in outbreaks of enteritis caused by *C. perfringens*. Cooked meat and smoked fish is often associated with *L. monocytogenes*, and poultry meat with campylobacteriosis. Botulism is a rare disease usually associated with the ingestion of inadequately processed home-preserved foods, particularly cured pork products, fermented fish, fish eggs or marine mammals, and low-acid foods, including vegetables. Histamine poisoning, which is rarely a serious disease, is typically associated with scombroid fish species. Raw milk is commonly identified as the vehicle of campylobacteriosis, brucellosis, salmonellosis, and more recently enterohaemorrhagic *E. coli* infection. In addition, cheese made from raw milk has been the cause of listeriosis, brucellosis, staphylococcal intoxication and bloody diarrhea and hemolytic uraemic syndrome caused by enterohaemorrhagic *E. coli*.

The association between gastroenteritis caused by *Bacillus cereus* and temperature-abused cooked rice is well established. Undercooked ground beef is a vehicle of enterohaemorrhagic *E. coli* O157:H7 infections and other EHECs, although recent outbreaks have been associated with fresh produce, fermented meat products, contaminated water and unpasteurized dairy products. Produce such as raspberries and basil has been associated with outbreaks of cyclosporiasis, whereas contaminated water has been the principal vehicle of cryptosporidiosis. Fresh produce (especially berries), shellfish and RTE foods requiring extensive manual handling (e.g. sandwiches) are also closely associated with foodborne enteric viruses such as noroviruses and hepatitis A. Low-moisture foods, e.g., peanut butter, chocolate, tree nuts, cereals, chia powder, etc., have been commonly identified in outbreaks of salmonellosis.

8.3.2 Ecological Features

The primary source of foodborne microbial pathogens includes a variety of animal, human and environmental reservoirs. After contamination of food, behavior of the pathogens is influenced by the food composition, the presence of other microbiota and the environmental conditions of the food.

Many of the pathogens that affect man are widely distributed in the agricultural environment: for example, *Salmonella* spp., *Campylobacter* spp., *L. monocytogenes, Yersinia enterocolitica*, pathogenic *E. coli*, *C. perfringens* and *S. aureus*. Although foodborne disease has long been primarily associated with animal products (e.g., meat, poultry, seafood and dairy products), in recent years many large outbreaks have been traced to produce, including lettuce, sprouts, cantaloupes and raspberries (Lynch et al. 2009; McCollum et al. 2013). Man is also a reservoir of certain foodborne pathogens, some of which may persist for weeks or months in the carrier state, for example, *S.* Typhi, *Shigella* spp., hepatitis A and Small-Round Structured Viruses (SRSV) such as noroviruses (ACMSF 1995).

Particular food products present greater risk than others due to possible contamination during production and harvest, their intrinsic properties that affect microbial growth and survival, traditional preparation and handling practices specific to that food, and, often, the absence of a CCP that will eliminate the hazard. For example, foods consumed raw, such as oysters, present high risks to susceptible consumers as they may be contaminated with norovirus or *Vibrio vulnificus* at harvest. Ready-to-eat foods may be re-contaminated with *L. monocytogenes* that may grow during subsequent refrigeration unless there is a chemical(s) that inhibits growth or a competitive anti-listerial microflora in the product.

Local customs and standards of community hygiene, especially those related to food, water supply, and sanitation, are important determinants of the extent and variety of foodborne illnesses. Effectiveness of prevailing standards for safeguarding water supplies, milk supplies, and shellfish harvesting areas warrants consideration. Control of food-processing; detecting, recalling, or condemning contaminated foods; vermin control; public health supervision of food-service establish-

ments; and appropriate use of refrigeration in processing plants, food-service establishments, and homes all play a role in reducing the incidence of foodborne illness and influence the selection of sampling plans for particular commodities from particular sources.

Dietary customs specific to a region also influence the foodborne hazards. For example, the Japanese custom of eating undercooked chicken has contributed to the relatively high incidence of campylobacteriosis in that country (Takenouch 2016). Similarly, the various fermented traditional marine foods consumed among the native communities in Alaska and Canada contribute to the incidence of type E botulism in these regions (Leclair et al. 2013).

Members of the *Aeromonas hydrophila* group occur in raw fish, raw meats and other foods. Although high counts of *A. hydrophila* can occur in patients with various types of diarrhea, its role as a cause of foodborne diarrhea remains unclear. *Plesiomonas shigelloides* can be isolated from water and raw aquatic products. High numbers of *P. shigelloides* have occasionally been demonstrated in patients with diarrhea, but again its role in foodborne or waterborne illness remains in dispute.

8.3.3 Clinical Features

Certain foodborne microorganisms are inherently associated with severe illnesses in man. *C. botulinum* types A, B, E, and F, for instance, can produce toxins that cause neurological illness in healthy people, even when very small amounts are ingested. If not effectively treated with antitoxins and provided with respiratory support, the case-fatality rate may exceed 50%, although this is rare today, with rates typically being less than 5%. Virulence properties of *S.* Typhi, *S. dysenteriae* I, *V. cholerae*, certain strains of *S.* Typhimurium, enterohaemorrhagic *E. coli*, and *C. perfringens* type C enable these pathogens to cause severe disease, even death. Cholera may present a medical emergency because in malnourished cases 50–70% of dehydrated cholera patients die unless they are appropriately treated by oral or intravenous fluid and electrolyte replacement. *L. monocytogenes*, mainly affects susceptible people, typically pregnant woman, neonates, elderly and immunocompromised individuals, however, among these patients the mortality can be as high as 25%. People with underlying chronic disease, in particular males with a history of high alcohol consumption, are prone to infection by *V. vulnificus* that is associated with iron overload in the patient.

Initially, pathogenic *E. coli* were considered to be strains of specific O serogroups causing diarrhea, mainly in infants and referred to as the "classical" enteropathogenic *E. coli* (EPEC). However, several other types of *E. coli* have become recognized and are concerns of today's food industry. Enterotoxigenic *E. coli* is a major cause of infantile diarrhea in developing countries and a leading cause of traveler's diarrhea. Enteroinvasive *E. coli* (EIEC) closely resemble *Shigella* in pathogenicity and antigenicity. Enterohaemorrhagic *E. coli* (EHEC), such as *E. coli* O157:H7, were first identified as pathogens in 1982 and produce Shiga-like toxins. Food-associated outbreaks attributed to *E. coli* O157:H7 have been well documented and are of great concern because low doses (<100 cells) have a high probability of causing infection and the illness can be severe, sometimes leading to kidney failure and death, especially in young children and the elderly. It is also worth noting that there a number of non-O157 strains such as O26, O121, O45, O145 and O104 (enteroaggregative enterohemorragic strain) that have been involved in foodborne outbreaks.

Low doses ($10^1 – 10^2$ cells) of *S. dysenteriae* can cause shigellosis. The infectivity of other *Shigella* spp., *V. cholerae*, and some salmonellae may also be high in highly susceptible individuals such as infants, malnourished and immunocompromised persons. Also, the severity of enteritis caused by salmonellae, *Shigella* spp., and pathogenic *E. coli* is greater (and probably the infectivity higher) for the very young, the aged, immunocompromised and persons with concomitant diseases than in healthy young adults. In these groups of people, even the usually moderate gastroenteritis caused by *V. parahaemolyticus*, staphylococcal enterotoxin, *B. cereus*, or *C. perfringens* sometimes become severe.

The relatively rare instances where beta-hemolytic streptococci are foodborne may lead to tonsillitis complicated by severe sequelae of glomerulonephritis and arthritis, while cardiovascular disabilities (such as rheumatic fever) may follow. Convalescence after some other foodborne illnesses (particularly typhoid and paratyphoid fevers, brucellosis, and viral hepatitis) may be lengthy.

Many foodborne illnesses are associated with chronic secondary sequelae that can linger long after the acute effects of enteric infections. Examples include the Guillain-Barré syndrome, a rapidly ascending paralysis that can lead to death, which is in part associated with antecedent *C. jejuni* infections; reactive arthritis which follows enteric infections caused by *Salmonella, Shigella* spp., *Y. enterocolitica* and thermophilic campylobacters; hemolytic uremic syndrome which is associated with *E. coli* O157:H7 infection; depression from chronic diarrhea caused by *Toxoplasma*; and septic arthritis following salmonellosis.

8.3.4 Diagnostic Considerations

Physicians' experience and laboratory procedures play a crucial role in diagnosing foodborne illness. For example, few physicians are likely to have previously encountered botulism; hence misdiagnoses may occur even when symptoms are typical or occasionally when the symptoms are very mild or appear similar to other illnesses. Laboratory isolation of specific pathogens is the only way certain enteric foodborne diseases can be diagnosed because the clinical syndromes of many of these diseases may be similar, e.g., bloody diarrhea may be symptoms of bacillary or amoebic dysenteries and Shiga-like toxin-producing *E. coli*.

When a previously unrecognized foodborne disease is reported by a laboratory, awareness often increases and other incidents are revealed. The recognition of foodborne campylobacteriosis and enterohaemorrhagic *E. coli* O157:H7 infection, and later other non-O157 serotypes, illustrate this point.

In public health and food laboratories, completely satisfactory methods are not yet available for the routine isolation or detection of several foodborne pathogens from foods, such as *Shigella* spp., *Y. enterocolitica,* enterohaemorrhagic non-O157 *E. coli*, *Cyclospora, Cryptosporidium,* and foodborne viruses. Laboratory methodology, therefore, limits the sensitivity, accuracy and precision with which their presence can be measured (see Chap. 10).

8.4 Categorizing Microbial Hazards According to Risk

In this book, the term hazard is limited to microbiological concerns and are associated with foodborne illness. These are bacterial pathogens and their associated toxins or toxic metabolites, viruses, parasites and toxigenic fungi. The risks associated with microbial hazards vary greatly, ranging from quite mild symptoms of short duration to very severe, life-threatening illnesses. When deciding on the level of concern, health hazards generally fall into three categories:

8.4.1 Moderate Hazards

Moderate hazards are rarely life-threatening, do not result in sequelae, are normally of short duration, and cause symptoms that are usually self-limiting but can result in severe discomfort. Some microorganisms can be both severe hazards for specific populations and mild hazards for the general population. For example, *L. monocytogenes* can cause abortion and/or stillbirths in pregnant women, life-threatening disease among immunocompromised people, but cause no symptoms or only a mild flu-like illness and/or diarrhea of short duration in the general population.

8.4.2 Serious Hazards, Incapacitating, But Not Life-Threatening

These hazards result in disease of moderate duration, and do not normally cause sequelae. Some pathogens such as *C. jejuni* and other thermophilic campylobacters occur most commonly in the lower, moderate category of hazard, but some strains of *C. jejuni* cause severe illness, i.e., Guillain-Barré Syndrome (GBS) in susceptible persons. One case of GBS is estimated to develop per 2000 *C. jejuni* infections, typically 2–3 weeks post-infection. The majority of strains cause only mild diarrhea of moderate duration.

8.4.3 Severe Hazards, Life Threatening

These microbial hazards can result in substantial chronic sequelae or the effects can be of long duration, can affect either the general population, or may be specific to populations at high risk. Factors influencing the development of illness in high-risk populations include specific host susceptibility to infection such as listeriosis in pregnant women, cultural practices such as consumption of potentially hazardous foods unique to specific subpopulations, or to geographic influences such as fumonisin intoxication associated with regions in which moldy maize is consumed. About 5–10% of cases of acute infection due to *E. coli* O157:H7 go on to develop hemolytic uremic syndrome (HUS) and patients who develop HUS have a mortality rate of around 3–5%.

The major microbial pathogens and toxins associated with foods in relation to their impact to public health, their frequency of involvement in disease, the types of foods that have served as vehicles, and significant factors contributing to disease, are listed in Table 8.2. This table is not intended to be all-inclusive and no attempt has been made to arrange these pathogens and toxins according to frequency with which they cause outbreaks or cases of foodborne illness, because this varies with locality. Table 1.1 (Chap. 1) indicates whether microbiological testing of foods (e.g., at a port of entry) or other control measures have been instrumental in controlling the hazard and ensuring food safety.

Table 8.2 Ranking of foodborne pathogens or toxins into hazard groups (severity of threat to health)

Microorganism	Frequency of involvement in foodborne disease	Examples of vehicles associated with outbreaks	Other factors contributing to significance
I. Moderate, not usually life-threatening; no sequelae; normally short duration; symptoms are self-limiting; can be severe discomfort			
Bacteria			
Arcobacter butzleri and *A. cryaerophila*	Low; the prevalence of *Arcobacter* infection may be underestimated and the exact routes of transmission are unknown.	Contaminated foods of animal origin (particularly poultry and pork) and consumption of contaminated water are likely to be the most important transmission routes.	Patients can be asymptomatic. Symptoms associated with acute watery diarrhea, sometimes being persistent or recurrent for greater than 2 weeks or even as long as 2 months. Antimicrobial therapies may be effective with resolution within few days.
Bacillus cereus (*B. cereus* gastroenteritis) including emetic toxin	Common	Fried and boiled rice; reconstituted cereal products; puddings, custards	Usually diarrhea and/or vomiting of short duration; death is rare
Campylobacter jejuni [a]	High; a leading cause of bacterial diarrheal illness in the US, often implicated with raw poultry.	Resident in the intestinal track, reported high contamination in raw poultry as well as with raw milk and untreated water.	Symptoms includes diarrhea, which may be watery or sticky, contain blood, lasting several days, but most are self-limiting.

(continued)

Table 8.2 (continued)

Microorganism	Frequency of involvement in foodborne disease	Examples of vehicles associated with outbreaks	Other factors contributing to significance
Clostridium perfringens type A (*C. perfringens*)[a]	High; the third most common foodborne disease in US and UK.	Spores of *C. perfringens* can withstand cooking temperatures and grow with improper refrigeration. Associated products include cooked, non-cured meats, poultry, and gravy.	Symptoms usually mild, but are more serious in elderly or debilitated persons; death is uncommon.
Enteropathogenic *Escherichia coli* (EPEC) [a], and Enterotoxigenic *E. coli* (ETEC) [a]	High; EPEC and ETEC are the leading bacterial cause of diarrhea in the developing world, as well as the most common cause of travelers' diarrhea.	Foods handled by persons carrying EPEC or ETEC; foods contaminated with non-potable water.	Diarrhea remains a leading cause of death in children younger than 5 years globally, especially in the developing world. For the general population, diarrhea is short in duration.
Staphylococcal enterotoxins (*S. aureus*) (enterotoxicosis or food poisoning)	Medium; frequently found in the human respiratory tract and on the skin, some are pathogenic whilst others are capable of producing heat-stable enterotoxins.	Cooked foods handled by persons carrying *S. aureus* then temperature-abused; ham; fermented sausages; cereal-filled pastries; cheese; milk, salads, peeled crustaceans, bivalve mollusk, and mushrooms.	Explosive vomiting and moderate diarrhea; symptoms usually resolve without treatment within 2 days of onset; death is rare.
Vibrio cholerae non O1 and non O139 (e.g., *V. cholera* O75)	Low; sporadic; natural bacterial reservoir in sea and coastal waters, with seafood as vector for transmission for the disease.	Primary transmission through consumption of raw or undercooked seafood, particularly bivalve mollusks, cross-contaminated cooked crustaceans.	Mild diarrhea to severe watery diarrhea not typically associated with fever or bloody diarrhea. Treatment primarily by rehydration, and antibiotic therapy.
Vibrio parahaemolyticus (*Vibrio parahaemolyticus* gastroenteritis)	Medium; common, natural bacterial reservoir in sea and coastal waters with seafood as vector for transmission for the disease.		
Parasites			
Cryptosporidium parvum[a]	Medium; *Cryptosporidium* pose a risk to rural environments where water supplies are untreated and susceptible to contamination from agricultural animal waste/manure, animal wastewater, septic tank effluents and septage.	Untreated water, unpasteurized apple juice, contaminated produce, and unpasteurized milk.	Cryptosporidiosis is typically an acute, short-term infection, but can become severe in children and the immunocompromised.
Cyclospora cayetanensis	Medium; infection can occur by consuming food or water contaminated with the parasite. People living or traveling in countries where cyclosporiasis is endemic may be at increased risk for infection.	Humans are the only host with infection transmitted through the fecal-oral route. Foods associated include berries especially raspberries (fresh and frozen); lettuce; basil; snow peas, cilantro, and water.	Gastroenteritis with a persistent watery diarrhea lasting over several days to weeks before self-limiting. Unless treated, illness may relapse with increase severity for immunocompromised people.
Giadia lamblia	Medium; common in developing countries, but low in developed countries. Giardiasis is most frequently associated with the consumption of contaminated water.	Contaminated water, vegetables, and food contamination by infected or infested food handlers.	Infection can be asymptomatic. Diarrhea within 1 week of cyst ingestion with illness lasting for 1–2 weeks, with some chronic cases lasting from months to years.

(continued)

Table 8.2 (continued)

Microorganism	Frequency of involvement in foodborne disease	Examples of vehicles associated with outbreaks	Other factors contributing to significance
Toxoplasma gondii [a]	Medium; infection (close to 30% of the world population may be infected with *Toxoplasma*) but with sporadic illnesses.	Undercooked, contaminated meat (especially pork, lamb, and venison) with cysts, any food or water contaminated with oocycsts, or through contacts with cat feces that contain *Toxoplasma*.	Although mild, flu-like symptoms occasionally occur during the first few weeks following exposure, infection are usually asymptomatic in healthy people.
Trichinella. spp.	Medium; sporadic and occasional outbreaks. Most of the infections in Europe, with about half from Romania.	Pork (from domestic pigs and wild boars), horse meat, wild game meat.	Acute stage with general weakness, chills, headache, fever (up to 40 °C), excessive sweating and tachycardia followed by symmetrical eyelid and periocular oedema.
Viruses			
Caliciviridae, including norovirus and sapovirus	High: can be found in human and other animal host, with transmission generally by the fecal-oral route, but can also be transmitted via the respiratory route.	Faecally contaminated raw bivalve mollusks and transmission of food handled by infected persons, leafy vegetables, fruit (especially berries) and mollusks.	Commonly cause acute gastroenteritis which may include vomiting and diarrhea. Symptoms emerge after an incubation time of 2 days and the symptoms only generally last for 3 days.
Hepatitis E virus[a]	Low; found worldwide, but with the highest prevalence in East and South Asia and Sub-Saharan Africa and in developing countries.	Contaminated water; sporadic cases linked to raw or undercooked pig liver/ sausages.	Typical signs and symptoms of hepatitis include jaundice, loss of appetite, an enlarged, tender liver, abdominal pain and tenderness, nausea and vomiting as well as fever. In rare cases, acute hepatitis E can result in acute liver failure and death. Overall population mortality rates from hepatitis E range from 0.5% to 4.0%.
Others			
Biogenic amines (e.g., histamine)	Rare; histamine is produced and can accumulate when bacterial enzymes metabolize naturally occurring histidine in fish or in the fermentation of some food products.	Scombroid fish, fermented sausages and fermented fish meat and some cheeses.	May occur when fish is held at ambient or high temperatures or when fermented food products have availability of free amino acids, the presence of microorganisms producing biogenic amines enzymes.
II. Serious hazard; incapacitating but not life-threatening; sequelae infrequent; moderate duration			
Bacteria			
Listeria monocytogenes[a]	Low; sporadic, making epidemiological links to food can be difficult. The capability for growth at low temperatures permits multiplication in refrigerated foods.	Soft and semi-soft cheeses, deli-meats, paté, smoked fish, fermented raw-meat sausages, produce (e.g., cantaloupe, cut celery, sprouts)	Its ability to grow at temperatures as low as 3 °C permits multiplication in refrigerated foods. Low numbers of *L. monocytogenes* are often consumed on a wide range of RTE foods.
Salmonella Enteritidis, *Salmonella* Typhimurium and other *Salmonella* serovars[a] (salmonellosis)	High; widespread occurrence in animals, especially in poultry and swine and environmental sources including water, soil, insects, factory surfaces and food preparation surfaces.	Eggs; poultry; produce, dairy products; wide range of other foods, including low-moisture foods	Can be serious for young and elderly persons; cross-contamination from raw meat and poultry; eggs and poultry meat can be internally contaminated during production; some serovars of *Salmonella* are highly virulent; reactive arthritis occurs in 1–2% of cases (Reiter's syndrome)

(continued)

Table 8.2 (continued)

Microorganism	Frequency of involvement in foodborne disease	Examples of vehicles associated with outbreaks	Other factors contributing to significance
Shigella flexneri, S. boydii, S. sonnei (shigellosis) (non-dysentery)	Low; sporadic in industrialized countries, sometimes endemic in developing countries.	Fecally contaminated water and unsanitary handling by food handlers are the most common causes of contamination. Foods subject to contamination includes salads, raw vegetables, milk and dairy products, and poultry.	Serious for young and elderly persons; secondary infections among contacts; sometimes low infectious dose, HUS occasionally.
Yersinia enterocolitica (pathogenic), *Yersinia pseudotuberculosis* (yersiniosis)	Rare; sporadic, does not occur frequently, unless due to loss of food manufacturing controls.	Yersiniosis is most often acquired by eating contaminated food, especially raw or undercooked pork products. Drinking contaminated unpasteurized milk or untreated water can also transmit the infection.	Most infections occur in children less than 5 years of age, with symptoms of mild gastroenteritis; in older children symptoms are severe, presenting a pseudo appendicular syndrome; only certain serovars and strains of *Y. enterocolitica* are pathogenic; sequelae; arthritis can occur in genetically-predisposed persons that carry the human leucocyte antigen (HLA-B27).
III.A. Severe hazard for general population, life threatening or substantial chronic sequelae or long duration			
Bacteria			
Botulinum neurotoxin (*Clostridium botulinum, C. butyricum, C. barati*) (botulism)[a]	Rare; associated with inadequately processed, home-canned foods, but occasionally commercially produced foods have been involved.	Improperly processed canned or preserved low acid foods: "home" cured meat products; smoked fish, other marine products; foil wrapped baked potato in salad, garlic in oil, and carrot juice.	Rapid recognition and treatment essential for patient survival; substantial mortality
Brucella melitensis, B. abortus, B. suis (brucellosis)	Low; transmission is by contact with infected animals or animal products contaminated with the bacteria (cattle, goats, pigs, and dogs, amongst others). Common in endemic areas.	Raw milk and raw milk cheese, especially from goats and sheep.	Brucella function as facultative intracellular parasites, causing chronic disease, which usually persists for life.
Coxiella burnetii (Q fever)	Rare; Q fever is a worldwide disease with acute and chronic stages caused by the bacterium *Coxiella burnetii*. Cattle, sheep, and goats are the primary reservoirs.	Human infections mainly result from the inhalation of dust contaminated with bacteria from the placenta and birth fluids or faeces from infected animals, and consumption of unpasteurized milk. Other modes of transmission, such as through contaminated water or the faeces of infected arthropods are rare.	Most people with acute Q fever infection recover, although others may experience serious illness. Pregnant women who are infected may be at risk for pre-term delivery or miscarriage. Chronic Q fever is a severe disease occurring in <5% of acutely infected patients.

(continued)

Table 8.2 (continued)

Microorganism	Frequency of involvement in foodborne disease	Examples of vehicles associated with outbreaks	Other factors contributing to significance
Enteroaggregative hemorrhagic *E. coli* (e.g., *E. coli* O104:H4) (hemorrhagic colitis and hemolytic uremic syndrome)	Low; emerging *E. coli* pathotype that is endemic in Central Africa, but can be sporadic in Europe and Asia.	One large outbreak, in Germany, was traced to raw sprouted seeds.	Very severe for children and elderly, severe complications including kidney failure and death, low infectious dose .
Enterohemorrhagic *E. coli* (e.g., *E. coli* O157:H7, O26, O111) (hemorrhagic colitis and hemolytic uremic syndrome)	Medium; hemorrhagic colitis infections are not too common, but may not be reflective of the true frequencies, with undercooked or raw hamburger implicated in outbreaks.	Undercooked ground beef, unpasteurized apple juice, vegetable sprouts, leafy greens, venison, yoghurt, fermented sausage, untreated and recreational water, contact with farm animals.	Very severe for children and elderly, severe complications including kidney failure and death, low infectious dose, acid tolerance.
Mycobacterium bovis (tuberculosis)	Rare in developed countries, but common in developing countries where milk pasteurization or cattle examination are not routine. *M. bovis* can cause tuberculosis in humans and other mammals.	Raw (unpasteurized) milk, raw milk cheeses.	Tuberculosis typically attacks the lungs, but can also affect other parts of the body. It is spread through the transmission of respiratory fluids through the air. If left untreated, may lead to high mortality.
Salmonella Typhi, *S.* Paratyphi A, B (*S.* Schotmulleri) and C (typhoid and paratyphoid fevers)	Medium, endemic in many parts of the world, occasionally epidemic.	Untreated water, raw milk, meat products, raw shellfish, and unpasteurized tempeh.	Prolonged medical care required, asymptomatic chronic carrier state commonly occurs
Shigella dysenteriae I (shigellosis)	Low; in developed countries. High; sporadic and endemic in developing countries where the spread is by contaminated water and food.	Fecally contaminated water and unsanitary handling by food handlers are the most common causes of contamination; salads, raw vegetables, and untreated water.	Severe dysentery due to potent Shiga toxin. High mortality rate, especially among children, low infectious dose.
Vibrio cholerae O1 and O139 (cholera)	Medium; cholera is generally a disease spread by poor sanitation, resulting in contaminated water supplies, but may also be transmitted by shellfish harvested from nonpolluted waters. Sporadic; endemic; sometimes epidemic	Raw seafood from polluted water; untreated water.	Substantial mortality among dehydrated, untreated persons; moderate symptoms with available rehydration treatment.
Parasites			
Taenia saginata	Medium: globally found to be most prevalently where cattle are raised and beef is consumed. Common in Africa, some parts of Eastern Europe, Southeast Asia, South Asia and Latin America.	Raw or undercooked beef or game meat containing live cysticerci.	Patients are either asymptomatic or suffer from non-specific symptoms like vomiting, nausea, epigastric pain, diarrhoea and weight loss, and in rare cases, ileus, ancreatitis, cholecystitis, cholangitis and an acute cholangitis.
Taenia solium (Cysticercosis)	Medium; an intestinal zoonotic parasite found throughout the world, and is most prevalent in countries where pork is eaten.	Raw or undercooked pork for adult tapeworm and fresh produce for cysticerci, the larval form of *T. solium*,	

(continued)

Table 8.2 (continued)

Microorganism	Frequency of involvement in foodborne disease	Examples of vehicles associated with outbreaks	Other factors contributing to significance
Others			
Abnormal Prions or Protease Resistant protein (PrP^{Sc})	Rare; sporadic diseases such as variant Creuzefeldt-Jacob disease.	Bovine offal of infected animals, where prions are accumulated. Those organs are target of regulation at slaughter as Specified Risk Material (SRM) (brain, spinal cord, intestines, tonsils, thymus, spleen).	Severe central nervous system disorder resulting in death; no treatment or cure.
III.B. Severe hazard for vulnerable populations, life-threatening or substantial chronic sequelae or long duration			
Bacteria			
Campylobacter jejuni serovar O19 and other serotypes associated with GBS (Guillain-Barré Syndrome) [a]	Low, infection with *Campylobacter jejuni* has emerged as one of the most common antecedent events associated with Guillain-Barré Syndrome.	Poultry, water, and raw milk.	Guillain-Barré Syndrome is an acute demyelinating polyneuropathy characterized by an immunologic attack upon peripheral nerve myelin.
Clostridium perfringens type C (enteritis necroticans) [a]	Rare, in developed countries, where it has been seen primarily in diabetics. Sporadically in parts of Asia, Africa, and the South Pacific, where it primarily affects children with severe protein malnutrition.	Cooked poultry and cooked pork	High mortality in protein-deficient persons, associated with malnutrition and a diet rich in trypsin inhibitors
Clostridium botulinum (types A and B) [a]	Rare; sporadic. Infant botulism results from the ingestion of the *C. botulinum* spores, subsequent colonization of the small intestine with release of neurotoxins.	Honey (infants under 1 year of age)	Infant botulism, if untreated, may progress to cause paralysis of the respiratory muscles, arms, legs, and trunk.
Cronobacter species *(C. sakazakii)*	Low; while *Cronobacter* infection can rarely occur in adults and children, neonatal and infant infections have been associated particularly with *C. sakazakii.*	Powdered infant formula (temperature abuse of dehydrated infant formula)	Causes death in infants <6 months of age (up to 70% mortality rate among neonates).
E. coli (EPEC and ETEC) [a]	Low; comprise a small proportion of enterovirulent *E. coli,* the species have been associated with diarrheal illness of all age groups from diverse global locations.	Untreated water; food contaminated by non-potable water or infected food handler.	Symptoms are mild, but can be severe in infants; major cause of infant mortality in certain regions; travelers' diarrhea.
Listeria monocytogenes [a]	Low; sporadic; occasionally epidemic.	Foods where multiplication has occurred during storage (see list above).	High-risk groups include immunocompromised persons and pregnant women; high mortality (ca. 25%) in high risk populations; infrequent illness in immunocompetent persons; low numbers of *L. monocytogenes* are frequently consumed in foods.
Salmonella spp.[a]	Rare; incidence salmonellosis among infants, higher than other age groups, has been associated with powdered infant formula.	Powdered infant formula and foods for infants <1 year of age.	Infants <1 year of age at risk for septicemia.

(continued)

Table 8.2 (continued)

Microorganism	Frequency of involvement in foodborne disease	Examples of vehicles associated with outbreaks	Other factors contributing to significance
Vibrio vulnificus[a]	Low, sporadic; present in marine environments such as estuaries, brackish ponds, or coastal areas.	Raw oysters	High mortality (ca. 50%) among persons that have elevated levels of serum iron; and those with liver disorders associated with high alcohol consumption.
Parasites			
Cryptosporidium parvum[a]	Medium; sporadic; endemic; occasionally epidemic.	Untreated water; unpasteurized apple juice, contaminated produce, and unpasteurized milk.	Severe prolonged diarrhea that is life-threatening in immunocompromised; prognosis is poor for AIDS patients; usually short term diarrhea that resolves spontaneously in immunocompetent persons.
Toxoplasma[a] *gondii*	Medium; infection (close to 30% of the world population may be infected with *Toxoplasma*) but with sporadic illnesses.	Undercooked, contaminated meat (especially pork, lamb, and venison) with cysts, any food or water contaminated with oocycsts, or through contacts with cat feces that contain *Toxoplasma*.	Usually asymptomatic in healthy people, but infection in pregnant women can result in fetal death, central nervous system abnormalities or eye disease in children. Severe toxoplasmosis could be developed in immunocompromised people.
Viruses			
Hepatitis A virus[a]	Medium; common in certain regions; severe disease more common in developed world.	Raw or underprocessed bivalve mollusks, produce (e.g., green onions, semi-dried tomatoes, frozen berries), untreated water.	Very severe for patients with liver disease, convalescence prolonged. Illness in adults more severe than in children. Lifelong immunity and vaccine available.
Hepatitis E virus[a]	Low; found worldwide, but with the highest prevalence in East and South Asia and Sub-Saharan Africa. Common in developing countries.	Contaminated water. Sporadic cases have been linked to raw or undercooked pig liver/ sausages.	Usually self-limiting, but may develop into acute liver failure. Pregnant women are at greater risk of obstetrical complications and mortality from hepatitis E, which can induce a mortality rate of 20% among pregnant women in their third trimester. Cases of chronic hepatitis E infection have been reported in immunosuppressed people.

III.C. Serious to Severe hazard for mycotoxins, acute or chronic dependent on exposure.			
Fungi	**IARC Classification[b]**	**Examples of vehicles associated with outbreaks**	**Other factors contributing to significance**
Aflatoxins, produced by *Aspergillus flavus*, *A. nomius* and *A. parasiticus* (aflatoxicosis)	Group 1, carcinogenic to humans.	Nuts, tree nuts, oilseeds, especially peanuts and maize, and dried figs in warmer climates.	Most potent liver carcinogens known; acutely toxic in high doses; carcinogenic, teratogenic and probably immunosuppressive at low levels.
Fumonisins, produced mostly by *Fusarium verticillioides* and *F. proliferatum*	Group 2B, possibly carcinogenic to humans.	Fungus endemic in maize, toxins present in staple diets in regions of high maize consumption.	Immunosuppressive, carcinogenic to rats and probably man, implicated in esophageal cancer.

(continued)

Table 8.2 (continued)

Ochratoxin A produced by *Penicillium verrucosum*, *Aspergillus ochraceus* and related species, *A. carbonarius, A. niger* and related species	Group 2B, possibly carcinogenic to humans.	Cereals especially wheat, rye and barley, and pig meats in cool temperate climates; dried fruit such as grapes, figs, dates, wines and coffee beans in warmer climates.	Nephrotoxic, probably contributing to reduced life spans in parts of Europe.
Trichothecene toxins, especially deoxynivalenol, nivalenol and T-2 produced by *Fusarium graminearum, F. culmorum* and related species	Group 3, not classifiable as to carcinogenicity.	Cereals especially wheat and maize in temperate climates.	Immunosuppressive, probably contributing to increased disease incidence in endemic areas.
Zearalenone, produced by *Fusarium graminearum* and related species	Group 3, not classifiable as to carcinogenicity.	Cereals especially wheat and maize in temperate climates.	Estrogenic effects, not commonly observed in humans.

Run rules
Severity
I. Moderate – not usually life-threatening; no sequelae; normally short duration; symptoms are self-limiting; can be severe discomfort
II. Serious hazard – incapacitating but not life-threatening; sequelae infrequent; moderate duration
III.A. Severe hazard – for general population, life threatening or substantial chronic sequelae or long duration
III.B. Severe hazard for vulnerable populations – life-threatening or substantial chronic sequelae or long duration
III. C Serious to Severe hazard for mycotoxins – acute or chronic dependent on exposure.
Evaluation of mycotoxins in humans (IARC 1993, 2002)

Mycotoxin	**International Agency for Research on Cancer evaluation**
Aflatoxins B and G	Group 1, carcinogenic to humans
Aflatoxin M_1	Group 2B, possibly carcinogenic to humans
Ochratoxin A	Group 2B, possibly carcinogenic to humans
Fumonisins	Group 2B, possibly carcinogenic to humans
Deoxynivalenol	Group 3, not classifiable as to carcinogenicity
Zearalenone	Group 3, not classifiable as to carcinogenicity

Frequency		
1.	Rare	Of an event, situation, or condition not occurring very often.
2.	Low	Occurring at irregular intervals or only in a few places; scattered or isolated.
3.	Medium	Of an event, situation, or condition found in relatively large numbers
4.	High	Widespread occurrence of an infectious disease in a community, common world wide

[a]Severity of disease varies for different subpopulations, thus this organism is listed in more than one category. See text for further information

[b]Evaluation of mycotoxins in humans, International Agency for Research on Cancer Evaluation (1993, 2002)

8.5 Definition of Cases

The foregoing information can be used to establish sampling plans that consider the risk associated with a hazard. Thus, the choice of a sampling plan must consider:

- significance of the test result in relation to the type and severity of disease (including effects in vulnerable groups), indicator of a microbial hazard or its commercial utility
- conditions under which the food is expected to be handled and consumed after sampling.

Table 8.1 classifies 15 different cases of sampling plans on a two-dimensional grid taking into account these factors. In the table, the stringency of the sampling plan increases with the type and degree of hazard: from a situation of no health hazard but of utility only, through a low indirect health hazard (as implied by the presence of indicator microorganisms), to direct health risks related to disease of moderate or severe implication. The stringency of the sampling plan also changes according to the conditions under which the food is expected to be handled. Hazards may remain unchanged, be reduced by cooking, or increase because of subsequent growth of microorganisms. The most lenient plan is case 1. Stringency increases from left to right and from top to bottom of the table, so that case 15 is the most stringent.

8.5.1 Factors Impacting the Case Choice

The choice of case depends on whether the hazard could increase, not change, or decrease between when a food is sampled (e.g., at port-of-entry) and when the food is consumed. Thus, the value of microbiological testing as a method of consumer protection depends on knowledge of the type of food to be sampled. For example, it is helpful to generally understand a food's normal method of production/harvesting, processing, composition, packaging, and the conditions to which it would normally be exposed during storage and preparation. In addition, some understanding of pathogen-food interactions and the intended consumer are needed. Information of this nature is needed before an examiner can choose an appropriate case. The following illustrates such considerations.

In general, foods that have received an adequate heat treatment during a manufacturing process are generally safer than those that have not. The risk increases when heat-treated foods (i) become contaminated after processing, (ii) are exposed to conditions that permit multiplication of pathogens, (iii) are not re-cooked shortly before consumption, and/or (iv) are targeted at vulnerable consumers.

If a food is expected to be fully cooked before consumption and because cooking reduces the hazard, one would choose case 4, 7, 10 or 13 depending on the degree of the hazard. Raw poultry, fresh dry pasta, cake mix, and dried soup mix are examples of foods in this category.

If conditions of anticipated use would not result in a change in the number of relevant bacteria (e.g., frozen storage), the appropriate case would be 5, 8, 11 or 14 depending on the type of hazard. Ice cream would be classified in one of these cases, because they are ordinarily maintained and consumed frozen.

If the food is ordinarily subjected to conditions that permit growth (e.g., *Salmonella* spp. in fresh-cut melons) or an increase in the hazard, thereby increasing risk, the case would be 6, 9, 12 or 15, depending on the type of the hazard (Table 8.1). One of these cases would also apply to dried milk since contaminating pathogens may multiply after reconstitution.

Preservation conditions The preservation conditions (e.g., salt concentration, a_w, pH, temperature) of the food should be considered in relation to the growth requirements of the relevant microorganism(s). Foods with a brine concentration of approximately 10% may support the growth of staphylococci but not salmonellae. Salmonellae, however, may survive for an extended period of time on dried meats. Hence, such products (if not refrigerated) might be classified in case 6 for staphylococci and case 11 for *Salmonella* spp.

Fresh meat supports the growth of various pathogens, whereas dried meat with a brine concentration of $\geq$ 16% in the water phase does not. Hence, if fresh meat is stored at temperatures allowing multiplication, the risk would increase corresponding to case 6, 9, 12 or 15, whereas for dried-beef there would be no change in risk and case 5, 8,11 or 14 would apply.

Storage temperature Temperature is especially important. Microbial numbers, and the associated risks, generally increase at 10–20 °C and even more rapidly at warmer temperatures. In contrast, refrigeration below 10 °C will control most hazards, because many pathogens do not multiply or do

so more slowly at low temperatures: for example, for ham kept below 6°C (at which temperature staphylococci do not produce toxin) case 8 rather than case 9 would apply. For foods in which psychrotrophic pathogens such as *L. monocytogenes*, *Y. enterocolitica* and non-proteolytic *C. botulinum* can multiply, the growth rate will decrease as the storage temperature gets closer to 0 °C. During storage at normal refrigeration temperatures (e.g., 4–7 °C) for a very limited period of time (1–3 days), limited or no growth can be assumed.

Competitive flora Growth of pathogens can sometimes be prevented by competition from other microorganisms. While salmonellae grow in most foods of appropriate pH, $a_{w,}$ and temperature, growth of staphylococci is often restricted by the associated spoilage microbiota. Fresh raw meats and bacon are not normally associated with staphylococcal food poisoning in part because they also carry large numbers of competing microorganisms that suppress the growth of *S. aureus*. The hazard of enterotoxin formation usually arises in foods that have been processed in some way to reduce the microbial population and then the food is contaminated with staphylococci (e.g., cooked ham contaminated after cooking).

Eating customs Custom also affects hazard and the choice of 'case.' For example, *V. parahaemolyticus* grows readily on raw fish unless it is refrigerated. It is a relevant cause of foodborne illness in Japan, where raw fish is commonly consumed, but in other countries, though widely distributed, *V. parahaemolyticus* is a much less common cause of illness because fish is cooked before consumption. Hence, for Japan case 8 or 9 would be appropriate for this pathogen, whereas in another country with different dietary customs, case 7 would be suitable.

Reconstituted dried foods Foods that are pasteurized before distribution (e.g., powdered eggs, dried milk) are sometimes eaten without cooking when distributed in relief areas. If a food is intended for consumers with unusually high susceptibility to foodborne illnesses, the hazard will be increased (Table 8.3).

Type of hazard Certain microbiological hazards can increase (e.g., salmonellae, *L. monocytogenes*) if the above conditions of temperature and food composition are favorable. Many hazards such as toxins and toxic metabolites tend to be quite resistant to environmental conditions, including normal cooking, and remain stable. Other hazards, such as viruses and parasites cannot increase in numbers, but may decline in concentration depending on the conditions to which they are exposed.

Table 8.3 Special foods for consumer groups with increased susceptibility

Food class	Reason for stringent sampling plan
Baby food	High susceptibility of the consumer population to enteric pathogens; severe response to infections and toxins; increased risk of fatality
Dietetic food	Infection is a severe risk for diabetics
Foods for hospitals, long-term care facilities	Patients may be prone to infection and to serious sequelae after enteric disease because of stresses from other disabilities and from immunosuppressive treatment, and under intensive care Interference with convalescence from other disease Staff and patients need to be protected because of their potential for spreading disease within the hospital
AIDS, transplant and cancer patients	Immunocompromised populations are highly susceptible to enteric pathogens
Relief foods, especially dehydrated high-protein foods	Populations needing relief foods are usually highly susceptible and prone to serious complications because of malnutrition and other stressful conditions. There is also increased risk for person-to-person spread of disease because of confinement of the population in crowded areas often having poor sanitary conditions. Particular hazards are reconstitution with contaminated water, unhygienic handling, and poor storage conditions leading to rapid bacterial growth

Susceptibility of the intended consumer If a food is intended for consumers with unusually high susceptibility to foodborne disease, the risk will be increased. Examples of special foods intended for high risk consumer groups (YOPI's young, old, pregnant and immunocompromised) are described in Table 8.3.

Storage and preparation for serving Only the usual conditions to which the food is expected to be exposed between when the lot is sampled and when the food is consumed should be considered. For example, a frozen food will ordinarily be kept frozen until it is cooked or reheated for serving. If a food is unexpectedly abused after having been sampled and approved (e.g., thawed under uncontrolled temperature conditions), the sampling plan may not provide the level of protection expected.

Method of food preparation An important consideration is the method of food preparation (e.g., normally eaten raw, warmed, baked, cooked).

8.5.2 Choosing Appropriate Cases: Examples

The following examples illustrate how knowledge about microbial ecology, as well as food storage and use are integrated in choosing the case:

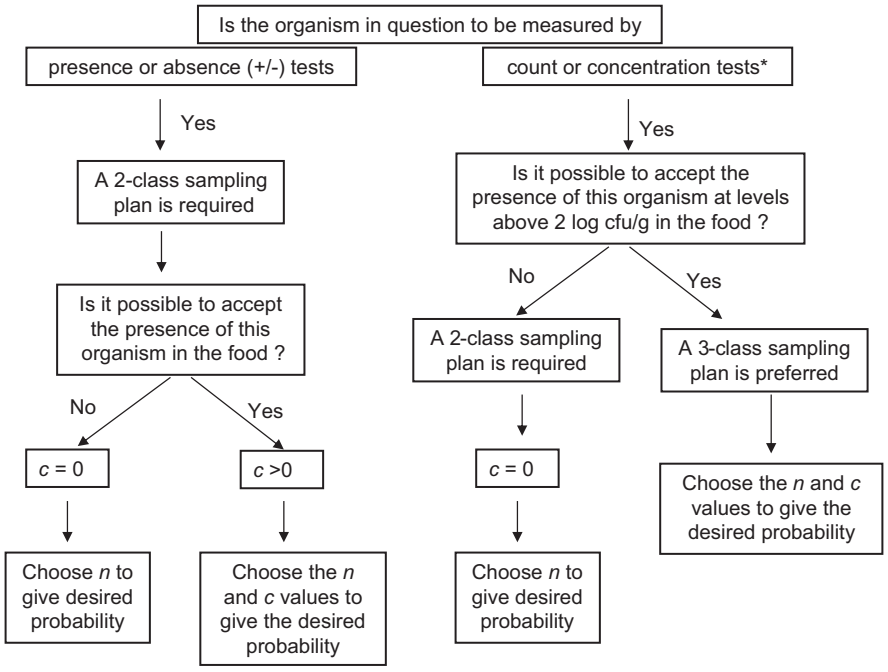
Salmonellae are serious hazards and often occur in raw protein foods (e.g., liquid eggs) but are inactivated by pasteurization. However, recontamination of pasteurized products with salmonellae can occur, and subsequent drying or freezing cannot be relied upon to destroy these bacteria. If such a dried food is consumed in the dry state, there is no change in hazard (case 11); if use after reconstitution is delayed, and heating does not take place before consumption (a practice that is highly undesirable with many such products), the case would be 12. Cooking promptly after reconstitution will reduce the hazard, hence case 10 would then be appropriate.

In a raw food (e.g., raw meat or poultry) that is to be cooked, testing for *S. aureus* would not be appropriate. If, however, the food has been cooked (e.g., cooked crustaceans or whole chickens) and then handled (e.g., peeling shrimp, removing skin and bones from the chickens), then contamination with *S. aureus* is a concern and if temperature abuse could occur case 9 would be appropriate and if storage temperature is well controlled case 8. In certain salted foods in which salt-tolerant *S. aureus* can grow, the competing flora is inhibited by the reduced a_w. Hence, the cases for staphylococci would be similar to a pasteurized food (case 9).

B. cereus and *C. perfringens* are also moderate hazards, differing from *S. aureus* in that they produce spores which survive mild heating. Few processes will reduce the hazard provided by these bacteria, so case 8 or 9 is usually appropriate. Consideration has to be given to subsequent use of the food. For example, if a dehydrated food is eaten immediately after reconstitution, then testing would not be appropriate, compared with a food for which a delay would be expected between when the food is rehydrated and when it is consumed (e.g., storage of leftovers or advanced preparation in catering).

The foregoing examples illustrate and emphasize the need for some knowledge of the microbial ecology and history of a food before an examiner can choose an appropriate case or even test for a particular purpose. When choosing cases on the basis of hazard as described above, one must consider the many possible uses for the consignment of food. Some uses may be of higher risk than others and the selection of case should reflect this possibility.

Microbial hazards are related to the presence of numbers of undesirable microorganisms or the occurrence and concentration of a toxic metabolite in a food. After choosing the category of hazard (category I, II or III in Table 8.2) and the effect of subsequent conditions of handling and preparing the food on the hazard, the appropriate case is selected (Table 8.1).


Is the organism in question to be measured by
presence or absence (+/-) tests
count or concentration tests*
Yes
Yes
A 2-class sampling plan is required
Is it possible to accept the presence of this organism at levels above 2 log cfu/g in the food ?
Is it possible to accept the presence of this organism in the food ?
No
Yes
No
Yes
A 2-class sampling plan is required
A 3-class sampling plan is preferred
c = 0
c >0
c = 0
Choose the n and c values to give the desired probability
Choose n to give desired probability
Choose the n and c values to give the desired probability
Choose n to give desired probability

*A variables plan may be applicable; see Chapter 7.

Fig. 8.1 Deciding between 2- and 3-class sampling plans

8.6 Deciding Between 2-Class and 3-Class Attributes Sampling Plans

To decide whether the plan should be 2-class or 3-class, one must consider whether any positives (e.g., *Salmonella* or APC levels above those reflected by GHP) can be permitted in any of the sample units. If the answer is no, a 2-class plan with c = 0 should be used. If the answer is yes, a 2- or 3-class plan can apply, but if the number of microbes in a unit-volume or mass can be obtained a 3-class plan is recommended (Fig. 8.1), for example for monitoring GHPs in slaughter.

Three-class sampling plans may, for the following reasons, be more appropriate than 2-class plans.

(a) To accept a proportion of sample units yielding test values in the marginally acceptable interval (between acceptable and defective), as these plans do, is in keeping with practical experience where, even under good manufacturing conditions, a few sample units may well reveal test values beyond those normally encountered without causing any consequent problem. This situation applies especially to counts of 'indicator' organisms.

(b) Sufficient experience also defines a different level, beyond which counts indicate substantial likelihood of health or utility hazard, a level that will not be attained if control has been adequate. This, designated M, should remain stable unless new experience reveals an error in its initial placement. Obviously, greater stability of criteria of acceptance may be expected to promote wider adoption of sampling plans.

(c) A 3-class plan allows both a regulatory authority and a food business to carry out a limited form of trend analysis. For example, an increasing proportion of values within the marginally acceptable (*m* to *M*) range may indicate a lack of control.

8.7 Determining Values for *m* and *M*

Definitions of *m* and *M* from a statistical point of view have been given in Chap. 7. From a microbiological point of view, the level of the test organism that is acceptable and attainable in a sample unit of a food is traditionally defined as being *m*. The value of *m* reflects implementation of Good Hygienic Practices (GHPs) by an importing country or, more often, for domestic production of a food, or is the hazardous level in 2-class plans. If the test organism is a pathogen for which there is no level of tolerance (i.e., for 2-class plans), *m* may be zero or, more correctly, absence in all analytical units when tested by a specified method. This will reflect the level of "detectability" in the test, e.g., < 0.04 cells per gram (<1 cfu per 25 g). Hence, the value of *m* for 2-class plans is usually 0 (in a specified quantity). For 3-class plans, *m* will usually be assigned some non-zero value.

M, used only for 3-class plans, is an unacceptable level. All sample unit results with values exceeding *M* are not considered acceptable (e.g., the lot should be withheld pending further investigation to determine whether the product can be used after further treatment or is unacceptable as food, or in other cases improvements to hygiene is defined as the action to be taken). Investigation of the processor's facilities should be undertaken when such lots are detected. In international commerce, this presupposes a system whereby the analytical results can be conveyed to appropriate officials of the country of origin.

There are several approaches to choosing the value of *M*:

1. As a utility (spoilage or shelf-life) index. Relate microbial levels to detectable spoilage (odor, flavor) or to a decrease in shelf life to an unacceptably short period.
2. As a general hygiene indicator. Relate levels of the indicator bacteria to a clearly unacceptable condition of hygiene – whether contamination or growth, or both.
3. As a health hazard. Relate levels of pathogens to illness. In choosing the value of *M* for a pathogen, use epidemiological and laboratory data in combination, experimental animal feeding or inoculation data, human feeding data, laboratory analyses for toxin related to levels of organisms, or other guides that indicate the level at which there is a serious concern for the hazard. For this purpose, consider the maximum amount of food likely to be eaten at one time and the susceptibility of the group of persons likely to eat the food (see also Table 8.3)
4. Knowing the distribution of the target bacterium in the food from studies one can use sampling plan tools to control by, for example, rejecting the worst 5% of the lots.

It is important to realize that *M* is defined by the hazard. The value of *m* is available and defined by GHPs in 3-class plans, and may change with time. In Chap. 7, a procedure for establishing a relationship between *m* and *M* is outlined. However, the values for *m* and *M* do not necessarily need to have a constant relationship.

In an unusual situation, one might find a consignment composed of two widely dissimilar lots. One lot may be entirely acceptable and the other entirely unacceptable (see for example curve 1 and curve 4 in Fig. 7.3; this situation would have a high proportion of units falling below *m* and above *M*, but few between *m* and *M*.

These examples illustrate how the choice of *m* and *M*, in relation to the microbial quality of the lot, influences the types of lots likely to be rejected. Moreover, they illustrate the importance to producers of limiting variation in the numbers of microbes present in food, i.e., of keeping production microbiologically 'under control', to avoid lots of otherwise satisfactory average quality being rejected. With

good or bad control, the average microbial levels are likely to be respectively low or high, and the distribution of the data around the average (expressed logarithmically) respectively narrow or broad. The spread of the distribution may be nearly as important as the average level, in determining compliance or non-compliance with a particular criterion, as indicated in Fig. 7.3.

The decision to accept or reject any particular lot, or other required action, should be based upon the laboratory results obtained, and is subject to the error associated with the laboratory procedure used (See Chap. 10). It is especially important when using 2-class plans with $m = 0$ and $c = 0$ that the methods used provide accurate results, since the occurrence of false-negative and false-positive results will lead to corresponding wrong decisions about lots because of the inaccurate methods.

Unfortunately, documentation of the inherent variation within a given method is rare, though experienced analytical microbiologists develop estimates that become included in their choice of criteria. Some methods, for example MPN methods for coliforms, have great inherent variability (Silliker et al. 1979). Similarly, it is common that familiarity of an analyst with a particular method (e.g., *Salmonella* spp. or *S. aureus*) enables that method to be most productive for them. This is a cogent argument for widely accepted use of a validated standard method so that all analysts become experienced in its use: another method with different sensitivity might require change of criteria. In specific cases the testing method is critical in establishing the acceptance criteria of sampling plans, and one should not deviate from a standard method unless there is a major advantage. In those cases the overall performance of the sampling plan should be set by selecting appropriate n and c values.

All probability computations in this book (e.g., operating characteristic (OC) curves and surfaces) for sampling plans assume that the laboratory results are obtained without error.

8.8 Specific Knowledge About the Lot

The relatively long delay between collection of samples and issue of the corresponding laboratory result may create a need for costly storage of product, or the implementation of other actions. For products with an extensive history of good quality, the need for storage can be avoided by releasing consignments as soon as samples have been taken (provided, of course, that records are adequate to trace for recall if a lot should prove to be unacceptable). If the analysis reveals an unsatisfactory condition, future consignments could be held at the port until the test results prove satisfactory. Upon re-establishing a satisfactory record (perhaps three consecutive consignments), the food could again be released as soon as samples have been taken. These systems have been in use by many countries.

Application of a sampling plan with a comparatively small number of samples (e.g., $n = 5$), and thus a low stringency, is only appropriate to detect lots with a high proportion of defectives. When a food product is processed and shipped under uniform, adequate controls and there is a favorable history of compliance and low risk associated with a defective lot that is not sampled, it could be argued whether acceptance sampling serves any purpose. Until such assurance has been obtained through a good history of test results and/or auditing (see Chap. 4), the only way to obtain satisfactory protection (i.e., substantial discrimination between good lots and unacceptable lots) is by increasing the sample size (larger n). However, as noted previously (Chaps. 6 and 7), to double the reliability may require four times as many sample units. The cost of the additional testing should be balanced against the potential gain in discriminatory power, hence a practical decision should be made. However, it should be balanced as well against the impact on the actual level of risk a wrong decision would have.

It may not be feasible to sample at random over the entire consignment. It may only be possible to sample randomly from a portion of the consignment from which the sample units can be drawn. If so, results apply only to the portion of the consignment sampled, not to the whole consignment, and it is necessary to consider whether the results can be applied to the entire consignment. For example, accessible containers may be those nearest the door of a vehicle, nearest the hatch of a ship's hold, or

on the periphery of a stack in a warehouse. When the contamination in a lot is truly random, the location of sampling of the entire lot is less important than when a non-random distribution is suspected. The sample units chosen may sometimes represent the portion of a lot or consignment that has been exposed to greater hazard, through contamination or conditions that permitted microbial growth. Convenient as they may be, they may also provide greater consumer protection through the selection from the portion of the lot most likely to be hazardous. This would not be so for a perishable food that is packed too warm and cools slowly in the boxes at the center of a pallet.

Another aspect that can only be addressed by referring to specific knowledge about the lot is the frequency distributions. As discussed in Chap. 7, knowledge of these distributions, especially their standard deviations, is needed to evaluate the performance of sampling plans based on quantitative criteria. The calculation of sampling plan performance, to be discussed in Sect. 8.11, assumes a standard deviation of 0.8. Frequency distributions having standard deviations above or below 0.8 will result in sampling plans that differ in their performance.

8.9 What Is a Satisfactory "Probability of Acceptance"?

Since decisions to accept or reject lots are made on samples drawn from the lots, occasions arise when the sample results do not reflect the true condition of the lot. The "producer's risk" describes the probability that an "acceptable" lot, if offered, will be falsely rejected. The "consumer's risk" describes the probability that a "bad" lot, when offered, will be falsely accepted. "Consumer's risk," for the purpose of this text, is considered to be the probability of accepting a lot whose actual microbial content is substandard as specified in the plan, even though the determined values indicate acceptable quality. This is expressed by the probability of acceptance (P_a) as given in Tables 7.1, 7.2 and 7.4. The "producer's risk" (the inverse of "consumer's risk") is expressed by $1 - P_a$.

When acceptance sampling is chosen to measure whether an FSO / PO is met, an additional uncertainty is introduced, i.e., the risk of incorrectly accepting a lot that does not meet the FSO/PO. Such an incorrect decision may increase consumer risk. In principle the minimally required probability to reject a lot not meeting the FSO / PO (or the confidence in appropriate operation of the sampling plan) should be adapted to the level of concern. This would lead to increasing minimal rejection probabilities with higher case numbers. The stringency of a plan is measured by the probability of accepting lots in which a particular proportion of sample units is defective. A relatively lenient 3-class plan ($n = 5$, $c = 3$) accepts a lot with a 5% proportion of defective units and 30% marginal units on about three occasions in four ($P_a = 0.75$). The most stringent 2-class plan ($n = 60$, $c = 0$) would accept lots with the same 5% proportion of defective units on about one occasion in 20 ($P_a = 0.05$), and lots with 0.5% defectives on about 15 occasions in 20 ($P_a = 0.74$).

If it is important to correctly determine whether a 0.5% proportion of defective units is in compliance with an FSO / PO, the question might be asked whether the plan can provide any worthwhile protection, even with a significant increase in the number of sample units. This demonstrates that microbiological testing will have very little value in process validation when the process is designed to produce a low proportion of defective units.

In practice, a probability of acceptance on three occasions in four ($P_a = 0.75$) means that one lot in every four will be rejected, a loss serious enough to compel a manufacturer to tighten microbial control to a level well below the limit(s) set in the test employed. Even the rejection of one lot in 20 ($P_a = 0.95$) may be sufficient to have this effect. Nevertheless, the protection conferred on the consumer for a particular lot is seriously limited when using small numbers of sample units like ($n = 5$); hence, the recommendation is to use large values for n when a direct hazard is recognized. In these situations, following testing and use of microbiological criteria will result in more reliable assurance of safety.

The sampling plan stringency must be considered in view of the associated criteria as well. Consider the example of a moderate health hazard, such as the presence of *S. aureus* in cooked peeled shrimp. If the microbiological limit *m* were placed at a numerical level well below that likely to represent hazard (*M*), one could frequently accept lots containing a high proportion of marginally acceptable units, which would require only a lenient sampling plan. If, however, *m* were placed nearer the hazardous level, one would accept such lots infrequently, requiring the use of a relatively stringent sampling plan. If *m* were at a hazardous level, one would not accept lots containing any units exceeding that level, and 2-class plans of high stringency would be required. Adjustment is possible by choosing limits known by experience to be associated with safety. When this is done, even though a high proportion of lots with substandard units will be accepted, the probability of consuming food that would cause illness is kept low.

8.10 Selecting *n* and *c*

The choice of *n* and *c* varies with the desired stringency (probability of rejection and power of discrimination) and hence with the cases in the grid of Table 8.4. For stringent cases *n* is high (until $n = 60$) and *c* is low (ultimately $c = 0$); for lenient cases *n* is low ($n = 5$) and *c* is high (until $c = 3$). As *n* decreases with the attributes plans proposed in this book, the chance of acceptance of unacceptable lots increases. This fact must be taken into account if the number of sample units (*n*) exceeds the analytical capability of a laboratory and *n* is reduced.

The procedure (see Chaps. 6 and 7) for selecting the number of sample units, *n*, should be first to fix desired acceptance and rejection probabilities for lots of defined acceptable and unacceptable qualities. Then the number of sample units required for this purpose is derived. However, the choice of *n* is usually a compromise between what is an ideal probability of assurance of consumer safety and the workload the microbiology laboratory can handle. Consider first the nature of the hazard, then decide the appropriate probabilities of acceptance and rejection for the hazard in question. Guidance

Table 8.4 Suggested sampling plans for combinations of degrees of health concern and conditions of use (i.e., the 15 "cases")

Degree of concern relative to utility and health hazard	Conditions in which food is expected to be handled and consumed after sampling in the usual course of events[a]		
	Conditions reduce degree of concern	Conditions cause no change in concern	Conditions may increase concern
Utility; general contamination, reduced shelf-life, incipient spoilage	Increase shelf-life Case 1 3-class n = 5, c = 3	No change Case 2 3-class n = 5, c = 2	Reduce shelf-life Case 3 3-class n = 5, c = 1
Indicator; Low, indirect hazard	Reduce hazard Case 4 3-class n = 5, c = 3	No change Case 5 3-class n = 5, c = 2	Increase hazard Case 6 3-class n = 5, c = 1
Moderate hazard; direct, limited spread	Case 7 3-class n = 5, c = 2	Case 8 3-class n = 5, c = 1	Case 9 3-class n = 10, c = 1
Serious hazard; incapacitating but not usually life threatening, sequelae are rare, moderate duration	Case 10 2-class n = 5, c = 0	Case 11 2-class n = 10, c = 0	Case 12 2-class n = 20, c = 0
Severe hazard; for (a) the general population or (b) restricted populations, causing life threatening or substantial chronic sequelae or illness of long duration	Case 13 2-class n = 15, c = 0	Case 14 2-class n = 30, c = 0	Case 15 2-class n = 60, c = 0

[a]More stringent sampling plans would generally be used for sensitive foods destined for susceptible populations

on determining these probabilities requires establishing a relationship between the 'confidence' desired and the impact a wrong decision (acceptance of a defective lot) would have on the actual risk. Determining the required number of sample units then depends on the desired confidence and the economic feasibility (CAC 2013).

The stringency of any of the sampling plans can be increased by adjusting *c* and/or *n*. In this way pressure can be brought to bear upon hygienic practices, nature of purchasing specifications, severity of processing, and extent and nature of the quality control practiced within the food industry concerned. The desirable effect should be carefully weighed, and the decision made should be known and understood by producer, manufacturer, distributor and control agency alike.

In summary, the final judgement on which a sampling plan is based should involve the relative weight to be placed on the above microbiological, epidemiological, and ecological factors, as well as the statistical probabilities of acceptance or rejection desired and economic considerations arising within the laboratory (e.g., the nature of its physical facilities, equipment, and work capacity of its personnel). Some degree of subjective judgement remains unavoidable because data adequate to allow fully objective decisions may not be available. Under these circumstances, individual judgements can vary widely, and if applied in international trade, could lead to widely differing sampling plans and inadvertent barriers to trade until the differences between trading partners are resolved.

8.11 Arithmetic and Geometric Means

Microorganisms grow (or decline) exponentially over time, and levels in food can vary over many orders of magnitude. Therefore, microbial numbers are often expressed as their log-transformed values. This has as advantage that numbers are easier to understand: 7.4 log cfu is comprehended better than 25,118,864 cfu. Additionally, errors in determination of microbial counts are often relative to their numerical ("absolute") concentration due to the enumeration methods used, which usually involve serial dilutions. A log transformation makes the errors more consistent in scale across very large difference in absolute numbers. This also has advantages for data analyses because most currently used statistics are only appropriate if the data conform to a 'Normal' distribution. The best available data suggest that the logarithm of microbial counts/concentrations in foods are consistent with the Normal distribution. Accordingly, log transformation of numbers/concentrations of microorganisms in foods is often used and is usually effective and appropriate in describing, interpreting and analyzing experimental results. This includes the design and interpretation of sampling plans that require that microorganisms in foods are "log-Normally" distributed (i.e. that the log cfu values are Normally distributed).

Conversely, the impact of organisms, including the public health risk from pathogens in foods, is most often related to the absolute number of organisms ingested. For example, at doses where probabilities of infection/illness are below 10% (i.e., below the ID_{10}), most dose-response models for infection/illness predict that the probability of illness/infection is directly proportional to the dose ingested. Therefore, in the interpretation of the public health impact the absolute number of organisms is most relevant.

A consequence of the logarithmic transformation of data is that that the average of log transformed values differs from the average of the untransformed values. For example, the average contamination level of three samples with 100, 1000 and 10,000 cfu/g present is 11,100 cfu/g /3 = 3700 cfu/g. The mean log contamination level is (2 + 3 + 4 log cfu)/3 = 3 log cfu/g. When converted to absolute numbers, this suggests a mean contamination level of only 1000 cfu/g. Importantly, in most situations, the risk from 3700 cfu/g is nearly four-fold higher than the risk from 1000 cfu/g.

As indicated above, it is the mean of the untransformed value that is most relevant to prediction of the effect of microbial loads. As such, while it is appropriate to calculate statistical summaries of

Table 8.5 Example of difference between geometric and arithmetic means for two batches with geometric mean concentration of 1000 cfu/g on linear scale and on log scale

Concentration in cfu/g							
	Product 1	Product 2	Product 3	Log mean	Geometric mean	Arithmetic mean	Total number
Batch 1	100	1000	10,000	3.568202	1000	3700	11,100
Batch 2	10	1000	100,000	4.527243	1000	33,670	101,010
Concentration in log cfu/g							
	Product 1		Product 2	Product 3		Mean log	
Batch 1	2		3	4		3	
Batch 2	1		3	5		3	

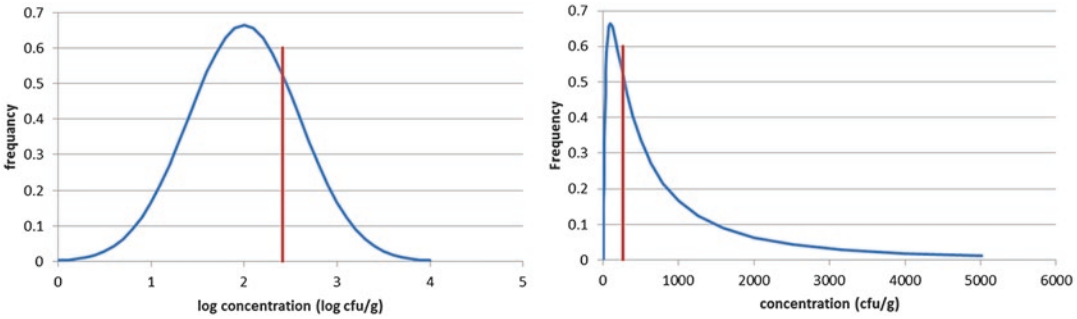


Fig. 8.2 Frequency of a lognormally distributed concentration with a mean log concentration of 2 and a standard deviation of 0.6. Panel A shows the normal bell shaped curve of log-transformed numbers; panel B shows the distribution of the actual concentration. The vertical line in both panels shows the arithmetic mean

microbial counts using log-transformed data (to facilitate the use of common statistical methods) it is the average of absolute values that is most relevant to estimation of risk.

Accordingly, it is important to specify clearly, and understand, the methods used in calculations and pay attention to the units used to express microbial loads, so that estimated risks can be compared without confusion.

As a second example, let's assume we have two groups of three products one with levels 100, 1000, and 10,000 and one with 10, 1000, and 100,000 (Table 8.5). If we consider these levels on log scale we can see that the log levels are for the first batch: 2, 3, and 4 log cfu/g and for the second batch: 1, 3 and 5 log cfu/g. Both batches have a mean log concentration of 3 log cfu/g. The second batch has higher variability, but based on the mean log concentration it has an equal level. If we now investigate these batches on arithmetic scale we see that the mean level is very different. The arithmetic mean of batch 2 is almost a factor 10 higher. This can also be seen in the log mean value of these batches. So the log mean gives a different result than the mean log. The geometric mean for both batches is 1000 cfu/g, resulting in the fact that the log (geometric mean) is equal to the mean log. This example illustrates the confusion when mixing log-transformed and actual numbers.

We can also investigate these effects if we would have distributions of microorganisms. Let's assume a batch with mean log concentration of 2 log cfu/g and a standard deviation of 0.6 log cfu/g (Fig. 8.2). We can see in the left graph the distribution of the log concentration which has the expected symmetrical bell shaped Normal distribution. If however the distribution of the actual concentration numbers is plotted we see an asymmetrical curve, where there is a long tail of high numbers. This results in the fact that the average on arithmetic scale (red line, log mean is 2.414) is more to the right than the mean on log basis (2).

The log mean ($\log(\bar{c})$) can be calculated from the mean log ($\overline{\log(c)}$) by the following formula:

Table 8.6 Cases and sampling plan performance assuming a standard deviation of 0.8. Lots having the calculated mean concentration or greater will be rejected with 95% probability

	Cases, sampling plans and calculation of their performance		
Type of hazard	Conditions reduce hazard	Conditions cause no change in hazard	Conditions may increase hazard
Indirect	**Case 4** (three-class, $n = 5$, $c = 3$) e.g. $m = 1000$/g, $M = 10{,}000$/g **Mean conc. = 5105/g (27,849/g)**	**Case 5** (three-class, $n = 5$, $c = 2$) e.g. $m = 1000$, $M = 10{,}000$ **Mean conc. = 3282/g (17,904/g)**	**Case 6** (three-class, $n = 5$, $c = 1$) e.g. $m = 1000$, $M = 10{,}000$ **Mean conc. = 1829/g (9976/g)**
Moderate	**Case 7** (three-class, $n = 5$, $c = 2$) e.g. $m = 1000$, $M = 10{,}000$ **Mean conc. = 3282/g (17,904/g)**	**Case 8** (three-class, $n = 5$, $c = 1$) e.g. $m = 1000$, $M = 10{,}000$ **Mean conc. = 1829/g (9976/g)**	**Case 9** (three-class, $n = 10$, $c = 1$) e.g. $m = 1000$, $M = 10{,}000$ **Mean conc. = 577/g (3147/g)**
Serious	**Case 10** (two-class, $n = 5$, $c = 0$) e.g. $m = 0/25$ g **Mean conc. = 18/1000 g (100/1000 g)** **1 cfu/55 g (1 cfu/10 g)**	**Case 11** (two-class, $n = 10$, $c = 0$) e.g. $m = 0/25$ g **Mean conc. = 5.6/1000 g (31/1000 g)** **1 cfu/178 g (1 cfu/33 g)**	**Case 12** (two-class, $n = 20$, c = 0) e.g. $m = 0/25$ g **Mean conc. = 2.0/1000 g (11/1000 g)** **1 cfu/495 g (1 cfu/91 g)**
Severe	**Case 13** (two-class, $n = 15$, $c = 0$) e.g. $m = 0/25$ g **Mean conc. = 3.0/1000 g (17/1000 g)** **1 cfu/328 g (1 cfu/60 g)**	**Case 14** (two-class, $n = 30$, $c = 0$) e.g. $m = 0/25$ g **Mean conc. = 1.2/1000 g (6.4/1000 g)** **1 cfu/854 g (1 cfu/157 g)**	**Case 15** (two-class, $n = 60$, $c = 0$) e.g. $m = 0/25$ g **Mean conc. = 0.5/1000 g (2.7/1000 g)** **1 cfu/2034 g (1 cfu/373 g)**

Concentrations given are geometrical means, with between brackets the corresponding arithmetic mean. The arithmetic mean can be calculated from the geometric mean and the standard deviation by: $\log(\bar{c}) = \overline{\log(c)} + 0.5\sigma^2 \ln(10)$

In this case ($\sigma = 0.8$) the arithmetic mean is $0.5 \cdot 0.8^2 \cdot \ln(10) = 0.7368$ logs higher, so a factor of 5.46 higher

The geometric mean is more informative for statistical calculations like for acceptance and rejection of lots, while the arithmetic mean might be more informative for the public health impact, since this represents the real average number of organisms in a lot

$$\log(\bar{c}) = \overline{\log(c)} + 0.5 \cdot \sigma^2 \cdot \ln(10)$$

A further explanation is provided in FAO/WHO (2016).

8.12 Sampling Plan Performance of the Cases

The cases described in Table 8.1 have been widely adopted in sampling plans. The ICMSF sampling plans were developed based on past experience, available data, practical constraints and statistical considerations and have provided helpful guidance. It should be noted, however, that the sampling plans assigned for each case in Table 8.4 may not provide the desired stringency required to come to a reliable decision for lot acceptance or whether a performance standard or FSO has been met. The plans simply imply that the number of samples indicated for each case provides the desired level of protection, whether this assumption holds must be assessed by the user.

The techniques described in Chap. 7 can be used to calculate the stringency of the cases in terms of the probability of accepting a lot at various concentrations of organisms. The stringency of each case and recommended sampling plan has an associated level of performance. Table 8.6 shows the performance of the sampling plans for cases 4 to 15. The table shows the mean concentrations that would be associated with an acceptance probability of 0.05 (i.e., a rejection probability of 0.95) for the sampling plan in each case. Both geometric and arithmetic mean are reported. To demonstrate the relative sensitivity of the 12 cases shown, constant values were used for m (10^3 cfu/g) and M (10^4 cfu/g)

Table 8.7 Cases and sampling plan performance assuming a standard deviation of 0.25, 0.5, 0.8 and 1.2. Lots having the calculated mean concentration or greater will be rejected with 95% probability. Reported means are **geometric** means with m = 0/25 g

	Cases, sampling plans and calculation of their performance		
Type of hazard	Conditions reduce hazard	Conditions cause no change in hazard	Conditions may increase hazard
Standard deviation of 0.25			
Serious	**Case 10** (two-class, n = 5, c = 0) **23/1000 g = 1 cfu/44 g**	**Case 11** (two-class, n = 10, c = 0) **11/1000 g = 1 cfu/93 g**	**Case 12** (two-class, n = 20, c = 0) **5.2/1000 g = 1 cfu/191 g**
Severe	**Case 13** (two-class, n = 15, c = 0) **7.0/1000 g = 1 cfu/142 g**	**Case 14** (two-class, n = 30, c = 0) **3.5/1000 g = 1 cfu/290 g**	**Case 15** (two-class, n = 60, c = 0) **1.7/1000 g = 1 cfu/585 g**
Standard deviation of 0.5			
Serious	**Case 10** (two-class, n = 5, c = 0) **21/1000 g = 1 cfu/49 g**	**Case 11** (two-class, n = 10, c = 0) **8.3/1000 g = 1 cfu/121 g**	**Case 12** (two-class, n = 20, c = 0) **3.6/1000 g = 1 cfu/275 g**
Severe	**Case 13** (two-class, n = 15, c = 0) **5.1/1000 g = 1 cfu/197 g**	**Case 14** (two-class, n = 30, c = 0) **2.3/1000 g = 1 cfu/433 g**	**Case 15** (two-class, n = 60, c = 0) **1.1/1000 g = 1 cfu/913 g**
Standard deviation of 0.8			
Serious	**Case 10** (two-class, n = 5, c = 0) **18/1000 g = 1 cfu/55 g**	**Case 11** (two-class, n = 10, c = 0) **5.6/1000 g = 1 cfu/178 g**	**Case 12** (two-class, n = 20, c = 0) **2.0/1000 g = 1 cfu/495 g**
Severe	**Case 13** (two-class, n = 15, c = 0) **3.0/1000 g = 1 cfu/328 g**	**Case 14** (two-class, n = 30, c = 0) **1.2/1000 g = 1 cfu/854 g**	**Case 15** (two-class, n = 60, c = 0) **0.5/1000 g = 1 cfu/2034 g**
Standard deviation of 1.2			
Serious	**Case 10** (two-class, n = 5, c = 0) **16/1000 g = 1 cfu/62 g**	**Case 11** (two-class, n = 10, c = 0) **3.2/1000 g = 1 cfu/308 g**	**Case 12** (two-class, n = 20, c = 0) **0.8/1000 g = 1 cfu/1198 g**
Severe	**Case 13** (two-class, n = 15, c = 0) **1.4/1000 g = 1 cfu/699 g**	**Case 14** (two-class, n = 30, c = 0) **0.4/1000 g = 1 cfu/2438 g**	**Case 15** (two-class, n = 60, c = 0) **0.1/1000 g = 1 cfu/7372 g**

Table 8.8 Cases and sampling plan performance assuming a standard deviation of 0.5 and 0.8. Lots having the calculated mean concentration or greater will be rejected with 95% probability. Reported means are **arithmetic** means with m = 0/25 g

	Cases, sampling plans and calculation of their performance		
Type of hazard	Conditions reduce hazard	Conditions cause no change in hazard	Conditions may increase hazard
Standard deviation of 0.25			
Serious	**Case 10** (two-class, n = 5, c = 0) **27/1000 g = 1 cfu/37 g**	**Case 11** (two-class, n = 10, c = 0) **13/1000 g = 1 cfu/79 g**	**Case 12** (two-class, n = 20, c = 0) **6.2/1000 g = 1 cfu/162 g**
Severe	**Case 13** (two-class, n = 15, c = 0) **8.3/1000 g = 1 cfu/121 g**	**Case 14** (two-class, n = 30, c = 0) **4.1/1000 g = 1 cfu/246 g**	**Case 15** (two-class, n = 60, c = 0) **2.0/1000 g = 1 cfu/496 g**
Standard deviation of 0.5			
Serious	**Case 10** (two-class, n = 5, c = 0) **40/1000 g = 1 cfu/25 g**	**Case 11** (two-class, n = 10, c = 0) **16/1000 g = 1 cfu/62 g**	**Case 12** (two-class, n = 20, c = 0) **7.1/1000 g = 1 cfu/142 g**
Severe	**Case 13** (two-class, n = 15, c = 0) **9.8/1000 g = 1 cfu/102 g**	**Case 14** (two-class, n = 30, c = 0) **4.5/1000 g = 1 cfu/223 g**	**Case 15** (two-class, n = 60, c = 0) **2.1/1000 g = 1 cfu/471 g**
Standard deviation of 0.8			
Serious	**Case 10** (two-class, n = 5, c = 0) **100/1000 g = 1 cfu/10 g**	**Case 11** (two-class, n = 10, c = 0) **31/1000 g = 1 cfu/33 g**	**Case 12** (two-class, n = 20, c = 0) **11/1000 g = 1 cfu/91 g**
Severe	**Case 13** (two-class, n = 15, c = 0) **17/1000 g = 1 cfu/60 g**	**Case 14** (two-class, n = 30, c = 0) **6.4/1000 g = 1 cfu/157 g**	**Case 15** (two-class, n = 60, c = 0) **2.7/1000 g = 1 cfu/373 g**
Standard deviation of 1.2			
Serious	**Case 10** (two-class, n = 5, c = 0) **730/1000 g = 1 cfu/1.4 g**	**Case 11** (two-class, n = 10, c = 0) **148/1000 g = 1 cfu/6.8 g**	**Case 12** (two-class, n = 20, c = 0) **38/1000 g = 1 cfu/26 g**
Severe	**Case 13** (two-class, n = 15, c = 0) **65/1000 g = 1 cfu/15 g**	**Case 14** (two-class, n = 30, c = 0) **19/1000 g = 1 cfu/54 g**	**Case 15** (two-class, n = 60, c = 0) **6.2/1000 g = 1 cfu/162 g**

Concentrations given as arithmetic mean. The arithmetic mean can be calculated from the geometric mean and the standard deviation by: $\log(\bar{c}) = \overline{\log(c)} + 0.5\sigma^2 \ln(10)$

In this case (σ = 0.8) the arithmetic mean is $0.5 \cdot 0.8^2 \cdot \ln(10) = 0.7368$ logs higher, so a factor of 5.46 higher

The geometric mean is more informative for statistical calculations like for acceptance and rejection of lots, while the arithmetic mean might be more informative for the public health impact, since this represents the real average number of organisms in a lot

Table 8.9 Cases and sampling plan performance assuming a standard deviation of 0.25, 0.5, 0.8 and 1.2. Lots having the calculated mean concentration or greater will be rejected with 95% probability. Reported means are **geometric** means with ***m* = 0/25 g**

	Standard deviation			
	0.25	0.5	0.8	1.2
Case 10 ($n = 5$, $c = 0$)	**23/1000 g = 1 cfu/44 g**	**21/1000 g = 1 cfu/49 g**	**18/1000 g = 1 cfu/55 g**	**16/1000 g = 1 cfu/62 g**
Case 11 ($n = 10$, $c = 0$)	**11/1000 g = 1 cfu/93 g**	**8.3/1000 g = 1 cfu/121 g**	**5.6/1000 g = 1 cfu/178 g**	**3.2/1000 g = 1 cfu/308 g**
Case 13 ($n = 15$, $c = 0$)	**7.0/1000 g = 1 cfu/142 g**	**5.1/1000 g = 1 cfu/197 g**	**3.0/1000 g = 1 cfu/328 g**	**1.4/1000 g = 1 cfu/699 g**
Case 12 ($n = 20$, c = 0)	**5.2/1000 g = 1 cfu/191 g**	**3.6/1000 g = 1 cfu/275 g**	**2.0/1000 g = 1 cfu/495 g**	**0.8/1000 g = 1 cfu/1198 g**
Case 14 ($n = 30$, $c = 0$)	**3.5/1000 g = 1 cfu/290 g**	**2.3/1000 g = 1 cfu/433 g**	**1.2/1000 g = 1 cfu/854 g**	**0.4/1000 g = 1 cfu/2438 g**
Case 15 ($n = 60$, $c = 0$)	**1.7/1000 g = 1 cfu/585 g**	**1.1/1000 g = 1 cfu/913 g**	**0.5/1000 g = 1 cfu/2034 g**	**0.1/1000 g = 1 cfu/7372 g**

Table 8.10 Cases and sampling plan performance assuming a standard deviation of 0.5 and 0.8. Lots having the calculated mean concentration or greater will be rejected with 95% probability. Reported means are **arithmetic** means with ***m* = 0/25 g**

	Standard deviation			
	0.25	0.5	0.8	1.2
Case 10 (n = 5, c = 0)	**27/1000 g = 1 cfu/37 g**	**40/1000 g = 1 cfu/25 g**	**100/1000 g = 1 cfu/10 g**	**730/1000 g = 1 cfu/1.4 g**
Case 11 (n = 10, c = 0)	**13/1000 g = 1 cfu/79 g**	**16/1000 g = 1 cfu/62 g**	**31/1000 g = 1 cfu/33 g**	**148/1000 g = 1 cfu/6.8 g**
Case 13 (n = 15, c = 0)	**8.3/1000 g = 1 cfu/121 g**	**9.8/1000 g = 1 cfu/102 g**	**17/1000 g = 1 cfu/60 g**	**65/1000 g = 1 cfu/15 g**
Case 12 (n = 20, c = 0)	**6.2/1000 g = 1 cfu/162 g**	**7.1/1000 g = 1 cfu/142 g**	**11/1000 g = 1 cfu/91 g**	**38/1000 g = 1 cfu/26 g**
Case 14 (n = 30, c = 0)	**4.1/1000 g = 1 cfu/246 g**	**4.5/1000 g = 1 cfu/223 g**	**6.4/1000 g = 1 cfu/157 g**	**19/1000 g = 1 cfu/54 g**
Case 15 (n = 60, c = 0)	**2.0/1000 g = 1 cfu/496 g**	**2.1/1000 g = 1 cfu/471 g**	**2.7/1000 g = 1 cfu/373 g**	**6.2/1000 g = 1 cfu/162 g**

Concentrations given as arithmetic mean. The arithmetic mean can be calculated from the geometric mean and the standard deviation by: $\log(\bar{c}) = \overline{\log(c)} + 0.5\sigma^2 \ln(10)$
In this case ($\sigma = 0.8$) the arithmetic mean is $0.5 \cdot 0.8^2 \cdot \ln(10) = 0.7368$ logs higher, so a factor of 5.46 higher
The geometric mean is more informative for statistical calculations like for acceptance and rejection of lots, while the arithmetic mean might be more informative for the public health impact, since this represents the real average number of organisms in a lot

Table 8.11 Cases and sampling plan performance assuming a standard deviation of 0.5 and 0.8. Lots having the calculated mean concentration or greater will be rejected with 95% probability. Reported means are **geometric** means with ***m* = 0/10 g**

	Standard deviation			
	0.25	0.5	0.8	1.2
Case 10 ($n = 5$, $c = 0$)	**57/1000 g = 1 cfu/18 g**	**51/1000 g = 1 cfu/20 g**	**46/1000 g = 1 cfu/22 g**	**40/1000 g = 1 cfu/25 g**
Case 11 ($n = 10$, $c = 0$)	**27/1000 g = 1 cfu/37 g**	**21/1000 g = 1 cfu/48 g**	**14/1000 g = 1 cfu/71 g**	**8.1/1000 g = 1 cfu/123 g**
Case 13 ($n = 15$, $c = 0$)	**18/1000 g = 1 cfu/57 g**	**13/1000 g = 1 cfu/79 g**	**7.6/1000 g = 1 cfu/131 g**	**3.6/1000 g = 1 cfu/280 g**
Case 12 ($n = 20$, c = 0)	**13/1000 g = 1 cfu/77 g**	**9.1/1000 g = 1 cfu/110 g**	**5.1/1000 g = 1 cfu/198 g**	**2.1/1000 g = 1 cfu/479 g**
Case 14 ($n = 30$, $c = 0$)	**8.6/1000 g = 1 cfu/116 g**	**5.8/1000 g = 1 cfu/173 g**	**2.9/1000 g = 1 cfu/340 g**	**1.0/1000 g = 1 cfu/975 g**
Case 15 ($n = 60$, $c = 0$)	**4.3/1000 g = 1 cfu/234 g**	**2.7/1000 g = 1 cfu/365 g**	**1.2/1000 g = 1 cfu/814 g**	**0.34/1000 g = 1 cfu/2950 g**

Table 8.12 Cases and sampling plan performance assuming a standard deviation of 0.5 and 0.8. Lots having the calculated mean concentration or greater will be rejected with 95% probability. Reported means are **arithmetic** means with ***m* = 0/10 g**

	Standard deviation			
	0.25	0.5	0.8	1.2
Case 10 ($n = 5$, $c = 0$)	**67/1000 g = 1 cfu/15 g**	**99/1000 g = 1 cfu/10 g**	**249/1000 g = 1 cfu/4 g**	**1824/1000 g = 1 cfu/0.55 g**
Case 11 ($n = 10$, $c = 0$)	**32/1000 g = 1 cfu/32 g**	**40/1000 g = 1 cfu/25 g**	**77/1000 g = 1 cfu/13 g**	**369/1000 g = 1 cfu/2.7 g**
Case 13 ($n = 15$, $c = 0$)	**21/1000 g = 1 cfu/48 g**	**25/1000 g = 1 cfu/41 g**	**42/1000 g = 1 cfu/24 g**	**163/1000 g = 1 cfu/6.1 g**
Case 12 ($n = 20$, c = 0)	**15/1000 g = 1 cfu/65 g**	**18/1000 g = 1 cfu/57 g**	**28/1000 g = 1 cfu/36 g**	**95/1000 g = 1 cfu/11 g**
Case 14 ($n = 30$, $c = 0$)	**10/1000 g = 1 cfu/98 g**	**11/1000 g = 1 cfu/89 g**	**16/1000 g = 1 cfu/63 g**	**47/1000 g = 1 cfu/21 g**
Case 15 ($n = 60$, $c = 0$)	**5.0/1000 g = 1 cfu/198 g**	**5.3/1000 g = 1 cfu/188 g**	**6.7/1000 g = 1 cfu/149 g**	**15/1000 g = 1 cfu/65 g**

and a standard deviation of 0.8 log cfu/g was assumed. The calculations indicate that lots having or exceeding the mean concentrations will be rejected with a probability of at least 0.95 when the indicated sampling plan is applied. Thus, the mean values define the sensitivity of each sampling plan.

The information for sampling plan performance provided in Table 8.6 enables the examiner to better understand the expected performance of a given sampling plan. Lots having the calculated mean concentrations or greater, as stated in this table, will be rejected with a > 95% probability.

For example, the most stringent sampling plan chosen for case 15 ($n = 60$, $c = 0$ with no positive samples detected ($m = 0$)) will, with 95% probability (and $\sigma = 0.8$), reject the lot if the geometric mean concentration of the pathogen is at least 0.5 per 1000 g (1 cfu/2034 g), or having an arithmetic mean of 2.7 per 1000 g (1 cfu/373 g). If lower mean concentrations of the pathogen are present (e.g., 1 in 5000 g), the sampling plan will accept the lot with a probability greater than 5%. In the sampling plan for case 8 (3 class-plan, $n = 5$, $c = 1$ with $m = 10^3$ and $M = 10^4$), a geometric mean concentration of at least 1829 cells per gram is required for the plan to reject the lot with 95% probability (and an arithmetic concentration of 9976 cells per gram). Other performances of sampling plans given various standard deviations and sampling weight of 25 and 10 g are included in Tables 8.7, 8.8, 8.9, 8.10, 8.11, and 8.12.

The poor performance of the sampling plans in detecting lots with low concentrations of pathogens demonstrates that lot acceptance testing is an unreliable approach to ensure consumer safety. It is for this and other reasons discussed throughout the text that greater emphasis should be placed on control systems, such as GHPs and HACCP.

8.13 The ICMSF Tool

The ICMSF has developed in 1998 a freely downloadable Excel spreadsheet, in which two-class and three-class sampling plans can be evaluated (Microbiological sampling plans: a tool to explore ICMSF recommendations http://www.icmsf.org/main/software_downloads.html). OC curves are presented both for the proportion defective samples and for the mean of the concentration distribution. The Log-Normal distribution is used to describe the microbial distribution. Furthermore the Poisson-log-Normal distribution is included for two-class sampling plans where the microbial method has an enrichments step (van Schothorst et al. 2009). Both geometric means and arithmetic means are reported. All quantitative data are easily available since it is a spreadsheet and the program is flexible in the type of scenario that needs to be evaluated.

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Chapter 9
Sampling, Sample Handling, Sample Analysis and Laboratory Quality Assurance

9.1 Introduction

Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of foods or ingredients for lot acceptance determination. Others may be for investigational purposes to assess control of the environment, investigate the source of a problem or to validate a process. Some may have legal implications relative to a lawsuit or for regulatory compliance. This chapter will discuss some of the major factors that should be considered when collecting sample units, shipping them to a laboratory, their preparation for analysis, analytical procedures, including laboratory quality assurance.

A sample unit is a small portion of the lot (batch) in which the distribution of both number and types of microorganisms should be representative of the whole lot (batch). This requirement is only completely fulfilled when the food and processing conditions result in a homogeneous distribution of microorganisms. This only occurs in well-agitated fluid products. In general the distribution of microorganisms is presumed to be random.

When cells are evenly distributed within the solid or liquid food, i.e., with a variance equal to the mean ($\sigma^2 = \mu$), the distribution approximates a Poisson distribution (Jarvis et al. 2012). However, more frequently cells occur in clumps or aggregates and their spatial distribution is erratic with the squared variance larger than the mean ($\sigma^2 > \mu$) representing, for example, a negative binomial distribution (Jarvis 2008).

The presence and distribution of pathogens, indicator organisms or spoilage microorganisms in finished products depends on many factors including the level and type of the initial microflora of raw materials, the structure of the raw materials, their quantities and ratio in recipes, and the processing conditions. Formulating and processing the food may also present certain microorganisms with opportunities to multiply in certain parts of the food (Wilson et al. 2002; Aguilar 2005) while others will be inhibited or even die. Post-process contamination, particularly when sporadic, may lead to the random presence of low levels of pathogens.

Representative samples can be collected from liquids such as milk in bulk if the product is thoroughly mixed before sampling. While adequate mixing can be achieved in small volumes, the efficiency of mixing decreases as the bulk volume increases. In the case of dry foods, difficulty in obtaining representative samples increases as the size of particles increases, e.g., from fine powders, such as milk powder or flour, to coarser particles, such as cereal grains, up to large particles or pieces such as nuts or raisins. Similar difficulties occur with meat, from whole carcasses, to large cuts, to trimmed pieces, to ground (minced) meat, and multi-component foods, such as prepared dishes with several ingredients.

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Similar limitations apply to samples from the processing line. Sampling in areas where residues accumulate, increases the probability of detecting deviations. For environmental samples, which should play an important role as an early warning of the ingress and colonization by pathogens, the situation is even more complex. Factors such as the complexity of the environment, the presence of niches where multiplication may occur, interactions with competitive microorganisms, and changing environmental conditions, such as relative humidity, temperature, or exposure to disinfectants, have an important impact on the distribution of pathogens such as salmonellae and *Listeria monocytogenes*. Another important factor is the small quantity of sample often available. This reinforces the need to have a well-designed sampling plan, appropriate sampling tools and well-trained personnel to collect the samples.

The distribution of microorganisms is also important when an analytical unit is withdrawn from the sample unit. Homogenization minimizes the heterogeneous distribution of microorganisms in the analytical sample, i.e., normalizes the distribution. As demonstrated using meat by Kilsby and Pugh (1981), the degree of homogenisation, from whole pieces to minced and finely chopped meat reduces the variance of counts from 0.334 to 0.075 and also increases the likelihood of detecting salmonellae.

Using the appropriate standard analytical methods or validated alternative methods, is a pre-requisite to obtaining reliable results. Several essential practical aspects, such as the need to adapt methods to certain food matrices to take into account the possible presence of injured microorganisms, the application of Good Laboratory Practices, etc., have an important impact on the successful detection or enumeration of the microorganisms.

The aim of this chapter is to review the different steps from sample collection to the analysis of the analytical unit, including laboratory quality assurance and to highlight potential problems that could affect the final result.

9.2 Collection of Sample Units

The purpose of sampling a food is usually to make a decision about the lot or to verify that the manufacturing processes are functioning as intended, based on the properties of the sample(s). Such decisions require the sample to represent the microbiological properties of the lot of concern (Legan and Vandeven 2003). Samples are collected throughout the whole food chain, serving different purposes depending on the information required. The needs of different parties such as regulatory authorities, food manufacturers, food retailers or researchers may be quite different. The reasons for sampling along the food chain, whether at the farm, at the processing facility, in a warehouse, at retail or even at the consumer's home, need to be considered. This facilitates selection of samples, correct interpretation of results and drawing valid conclusions.

Sampling may be for investigative purposes to obtain information and increase the knowledge on the occurrence of microorganisms in food products, or to assess the microorganisms behaviour during processing and storage. Regulatory authorities sample at predetermined points in the food chain to judge whether imported or commercially available foods comply with current legislation. Intensive sampling by the same authorities would be carried out during an outbreak to identify its source. In commerce, sampling is performed to confirm compliance with specifications between suppliers and customers. Samples taken at intermediate steps of the processing line, from surfaces of equipment in contact with the product or from the processing environment, allow the food processor to verify adherence to GHP and the efficacy of preventive measures. Sampling of end-product is often undertaken to verify the efficacy of the HACCP plan.

"Initial sampling" represents the step where physical samples are taken, according to a pre-established sampling plan, at specified sampling points. The sample unit is usually larger than the amount actually analyzed. "Sample handling" encompasses all subsequent steps including the description, recording and labelling of the samples, their intermediate storage before dispatch, their transport to the analytical laboratory, their reception, registration and storage up to the preparation of the analytical unit(s) before analysis. All steps up to the analysis must be controlled to ensure the quality of the sample units and allow reliable traceability, including "chain of custody" for regulatory samples. The guidelines provided in this chapter are considered good practice and should help optimize results.

9.2.1 Investigative Sampling

Investigative samples are generally collected to document observations, support regulatory actions or in response to an observed deviation or 'out of control' situations. In the latter case, one would most likely want to know the source of the problem and the extent of contamination so that appropriate action can be taken. Random sampling is not appropriate in this situation and the investigator should rather combine existing knowledge of microbiology, the process, equipment design and other relevant information to determine the sites and food that should be sampled, i.e., the implicated food and sites where the microorganism is most likely to be found (Legan and Vandeven 2003). It is important to ensure that samples that may be used as evidence in court are properly sealed, preferably with tamper-proof seals and their integrity and chain of custody protected. Dated photography of samples is a useful way to document integrity of samples prior to them leaving the facility. All documentation and/or photographs generated through this process should be maintained for an appropriate period of time in case any discrepancies or queries may arise. Random sampling is not considered an efficient approach to an investigation, which rely on targeted sampling (FAO 2008d; FDA 2011a). Further details can be obtained in Chap. 11.

9.2.2 Containers and Sampling Equipment

Unless sample units comprise products in their original packaging such as sachets, tins, boxes, bottles or cups, the samples drawn must be placed in clean, sterile containers. The containers used should be inert, of appropriate size, have a sufficiently wide opening to allow clean and easy transfer of the samples, and be able to maintain the integrity of the original sample. Depending on the sample unit, different containers may be used, such as plastic bottles, jars or bags, metallic cans or boxes. Glass containers, however, should not be used in manufacturing facilities where breakage could lead to the presence of glass in products.

Using sterile disposable containers is the most convenient, but for reasons of cost this may not be possible. If re-usable containers are used, they should be thoroughly washed, rinsed, dried and sterilized before further use. Depending on the material, containers are sterilized either at 121 °C for 15 min in an autoclave, or for at least 1 h at 160 °C in a hot air oven. Details of the different options for the sterilisation of containers can be found in APHA (2001).

It is important to prevent samples from leaking during handling and, in particular, during transport. Screw caps are the most appropriate closing devices for jars and bottles, and plastic bags must be sealed securely. If rubber or cork is used for caps or closures, it must be confirmed that no adverse interactions with the samples can occur.

Utensils used to collect the sample units should be sterile, and adapted to permit the most appropriate and representative sampling according to the type of food. Sterile scissors, knives, saws, can-openers

and other tools can be used to open boxes, packs, sacks or cans. Samples of the actual food product can then be collected using sterile scalpels, tweezers, tongs, spatulas, syringes, scoops, spoons, triers, probes, drill bits, forks, cork-borers, pipettes, swabs or sponges, depending on the type of food sampled and sampling procedure. Other sampling tools and materials, such as borers, insertion tubes, sealable bags, sealing tape and an insulated ice box containing ice or dry ice to transport perishable samples, are often necessary. Further details can be found in FAO (2008d).

Where temperature of the food is taken at sampling, thermometers should be of a digital electronic type rather than made of glass, to avoid breakage and consequential potential contamination. Thermometers should be sanitized by dipping in a hypochlorite solution at a minimum concentration of 100 mg/l for at least 30 s prior to use (APHA 2001).

For environmental sampling, utensils need to be adapted to the sampling site, with no limitations to their type, especially for less accessible sites. Sponges or swabs are most suitable to sample food contact surfaces, floors or walls. Scrapers, scoops, spatulas or brushes are suited to collecting residues in cracks, crevices or underneath equipment. Plastic pipettes and syringes can be used to collect water in drains and air sampling devices to monitor the air. Containers and utensils should be packaged individually and be pre-sterilised, thereby avoiding the need for disinfection between samples. Disposable plastic gloves are recommended when collecting certain samples (e.g., sponge samples from equipment), a new glove being used for each sample. Sterilization of utensils by means of a torch, should be avoided, and may even be dangerous (e.g. risking explosions in dusty environments).

9.2.3 Sampling Procedures

The method of sampling should be adjusted to fit the purpose and take into account the environment e.g., dry versus wet environments. For lot acceptance determinations the intent is to collect sample units that are representative of the lot. To assess control of the processing environment, a routine procedure should be developed including sample sites and method of sampling. When investigating the source of a problem the sampling sites, foods, ingredients, times, frequencies, etc. are not predetermined and left to the discretion of the sampler. While it is desirable to make every effort to collect samples that fulfil the intended purpose, the safety of the individual collecting the sample and others who may be affected, takes precedence. Under dangerous circumstances, e.g. operating/moving parts of equipment, sample collection should not be attempted. Likewise, if the method of sampling can jeopardize the food and render it injurious to consumers, e.g., creation of foreign bodies, the sampling procedure should be changed or the sample should not be collected.

Samples should be collected by persons previously trained and instructed in the use of appropriate methods and, above all, aseptic techniques. The timing and sampling point in the food chain or a food process may be crucial for data interpretation. When regulatory authorities collect samples at a port-of-entry, timing is determined by the arrival of trucks or ships. Samples collected by authorities to verify compliance with legal requirements may be collected during storage, distribution, marketing or foodservice. Samples collected at different steps in the processing line, after production and packaging, before and after cleaning, allow food manufacturers to verify adherence to GHP and the efficacy of HACCP.

For packaged products sampling is straightforward and need only follow pre-established sampling plans. For UHT products, for example, routine samples are taken at defined intervals during processing, while “event samples” are taken after start-up, after stops in production, or after changes of paper rolls and strips to monitor and assess the impact of such events (Cordier 1990). In other circumstances packed units may be taken from pallets according to a pre-determined scheme.

If the packaging material needs to be opened to collect the sample unit, appropriate precautions must be taken to avoid contamination. External surfaces must be cleaned to remove dust and soil and, if multiple layers of packaging are used (e.g., bags of flour, sugar or other dry products) the outer layer(s) can be removed. This allows access to cleaner surfaces, which may then be disinfected, if

necessary, before opening or cutting. Sufficient quantities should be collected to allow additional analyses to be performed as necessary, or for unforeseen enquiries. If packages show signs of "blowing" (swelling), extra care should be exercised to avoid dissemination of contaminated material. In such circumstances the whole package should be sent to the laboratory.

Ideally, the food should be mixed before collecting the sample units but this may not be practical or possible. For liquid samples such as milk, ice cream mixes or beverages stored in vats or tanks equipped with stirrers, it is usually relatively easy to obtain representative samples. If there is no evidence of recent stirring and mixing, thorough mixing with a sterile ladle is recommended before collecting the sample. An additional sample should be taken as a temperature control, in which case the temperature at time of sampling and again at receipt at the laboratory, should be checked and recorded. In some foods, however, sampling specific components, sections or layers of multi-component products may be required. In such cases, any sign of non-uniform material, which includes differences in shape, size or color of particles in crystalline, granular or powdered solid substances, moist crust on hygroscopic substances, deposits of solid material or stratification in liquid products should be noted during the sampling procedure. Portions of the material that are non-homogeneous should be sampled separately (FAO 2010). Particular care should be exercised to achieve a good separation. Examples are provided in Table 9.1.

Table 9.1 Special considerations when sampling food commodities

Phase	State	Form	Food/ ingredient	Recommendations for sampling
Solid	Dry	Fine powder	Dry milk, cocoa powder	From centre of container unless problems suspected at the surface
		Large particles	RTE cereals, dry pasta	Select representative components and blend before drawing sample; may be rehydrated directly
		Blocks	Dates, sugar, cheese	If hard, use sterile hammer and chisel (also mortar and pestle) to draw sample; trier to draw samples from several locations
		Multi-component	Soup mix, spices	Entire unit or selected components drawn for sample
Solid	Moist	Particles	Ground meat	Multiple portions drawn from one or more packages or units
		Chunk	Tote of beef primal cuts	Selected pieces or liquid weep (drip) from large tote
		Blocks	Whole poultry, cheese	Whole bird rinsed in diluent or media; trier to draw samples
		Carcass	Beef	Prescribed suspected area swabbed or sponged for testing
		Sausage	Sliced cooked	Rinse or blend
			Fermented sausage	Selected areas for selected analyses e.g. test for *Staph. aureus* near the surface, and EHEC in the centre
		Multi-component	Cream dessert, stew	Selected areas for selected analyses e.g. test for *Staph. aureus* near or in cream filling; test for *C. perfringens* in liquid portion of the stew
Solid	Frozen	Particles	IQF peas / beans	Frozen sample either "stomached" or rinsed with diluent
		Blocks	Frozen egg	Collected with (sterile) funnel and drill
		Multi-component	Pizza	Sector sampled while frozen unless specific components tested
Liquid		Single phase	Water	Membrane filter kept in conditions that avoid death or multiplication
		Multiple non-miscible phases	Salad dressing	Well-mixed representative sample, or specific layer
		Homogeneous multiple phases	High calorie supplement	Representative weighed sample

RTE ready-to-eat, *IQF* instant quick frozen

Water (e.g. potable water, processing water) should be sampled from taps or built-in sampling devices after flushing sufficient water through those taps or built-in devices to obtain a representative sample. A sterile ladle or other means can be used for water or brine solutions in open systems. Addition of a neutralizing solution is necessary to neutralize disinfectant residues, if present. Sampling ports or taps directly installed on equipment such as tanks are usually sterilized *in situ* by means of steam. They require particularly careful attention (design, cleaning and sterilisation procedures) to avoid contamination of the product.

Air sampling in processing environments may be performed to assess the microbiological status of air. Many techniques have been described, from the simple passive collection using sedimentation plates to active air samplers based on impaction or impingement. Methods and available equipment, are discussed in further detail by Nieguitsila et al. (2011) and Yamamoto et al. (2011).

Sampling methods for the detection and enumeration of microorganisms on the surface of freshly slaughtered (red) meat animals are described in ISO 17604:2003 (ISO 2003d). The microbiological sampling can be carried out as part of the process control (and to verify process control) in slaughter establishments for cattle, horses, pigs, sheep, goats and game raised in captivity, risk-based assurance systems for product safety, and surveillance programmes for the prevalence of pathogenic microorganisms. This international standard includes the use of destructive and non-destructive techniques depending on the reason for the sample collection. It does not, however, consider the use of sampling plans. ISO 17604:2003 includes three annexes showing (a) sampling sites on the carcass, (b) requirements for microbiological examination and (c) a comparison of destructive and non-destructive methods.

9.2.4 Sample Labelling

Sample units must be clearly labelled and identified to permit sufficient traceability. This can be achieved by writing descriptive terms or numbering each sample unit directly on the container or a firmly attached label, making sure that the ink cannot be washed off. In addition, a sampling report should be prepared, with relevant details stating, for example, the time of sampling, sample site, particular observations on packaging, etc. The comments may vary widely depending on the purpose of sampling and what is to be learned from the analysis. The report should indicate the reason for sampling and, if known by the person collecting the samples, the types of analyses to be performed. If necessary, for example for legal reasons or in case of dispute, the report should be signed by the person responsible for the sample collection as well as by representatives of the parties concerned. In such instances, containers with the samples may be sealed with an official seal that makes tampering impossible.

9.3 Retention Samples

Samples should be retained by both manufacturers and laboratories. This can be done for different reasons. Manufacturers should retain samples for the duration of the shelf-life of the product as a reference in the case of possible complaints arising from the market place. Such samples would then be tested microbiologically, most often by relevant authorities or by an independent and accredited laboratory.

Laboratories should retain samples for mainly regulatory requirements, particularly when samples form part of an investigation of a foodborne disease outbreak. It may be necessary to refer back to such samples at a later stage for further testing. In this case, original sample material should be kept in a manner that would prevent deterioration, yet not in a way that might inhibit future analysis. For example all perishable food and all frozen foods should be kept frozen and other items, e.g., ambient, shelf stable canned or dried samples, held at temperatures not exceeding 40 °C. For legal continuity the samples should be kept in a secure place, sealed to ensure the integrity of the food and clearly identified (FSA 2000).

9.4 Intermediate Storage and Transportation

Collected sample units should be transported to the laboratory as promptly as possible. However, in certain situations intermediate storage cannot be avoided before they are dispatched, e.g., when daily dispatch is impossible. For environmental sampling, samples may be collected at regular intervals from specific sites over a period of time, assembled and then sent as a single dispatch to the laboratory. When samples are stored, the storage conditions must prevent changes, i.e., microbial growth or death. Storing dry or shelf-stable products or samples pose relatively few problems, although they must be stored so as to avoid moisture uptake or extremes shifts in temperature resulting in condensation. Wet and perishable samples are more delicate and need to be refrigerated or even frozen. Appropriate storage conditions must be established to ensure that the target microorganisms remain unchanged and avoid overgrowth of competing microbiota. Loss of viability during frozen storage and subsequent thawing can be a particular problem (e.g., *C. jejuni*, vegetative cells of *C. perfringens*).

For transportation, all samples must be packed to avoid breakage and spillage. Where necessary, containers should be protected by additional packaging material. Finished products, raw materials and environmental samples should be packaged separately to avoid possible problems of cross-contamination or shipped separately. Samples of perishable chilled or frozen products should be shipped in insulated containers to maintain the appropriate temperature with a refrigerant such as ice, dry ice or freezer packs. For particularly sensitive or important samples, temperature recorders or indicators should be used to record in-transit temperatures that could affect the analytical results. For frozen products this can be as simple as an ice cube in a sealed bag so that thawing and refreezing are immediately apparent upon receipt at the laboratory.

Where swabbing is used to take microbiological samples, the swabs need to maintain the integrity of the samples and ultimately the release of the target cells. It is also important that swabs are transported appropriately so that the numbers and proportions of microorganisms present should be the same when the swabs arrive at the laboratory as when first sampled. Swab transport systems should be selected according to their ability to keep the more delicate and fastidious bacteria viable while preventing competitive microbiota from multiplying and obscuring others. An appropriate transport medium should be selected for this purpose (Rapidmicrobiology 2011).

9.5 Reception of Samples

When received at the analytical laboratory, sample units should be inspected visually for damage or spillage, temperature checked as necessary, and the samples cross-checked against the sample report. Information that could influence the analytical results should be noted on the sample report. Today Laboratory Information Management Systems (LIMS) are widely used, allowing for easy and complete registration, thus ensuring good traceability.

9.6 Sample Analysis

To ensure reliable and accurate analysis, a microbiologist must remove a representative subsample from the food sample received by the analytical laboratory and subsequently handled during the analysis in a manner wherein the target microorganism(s) remains unchanged in numbers and characteristics. How well this task is performed reflects the statistical reliability of the sampling procedure (see Chap. 7) and good laboratory practice that allows sufficient attention to the essential conditions required at all stages of analysis. These include media selection, formulation and preparation, sample storage and preparation for analysis, incubation conditions for resuscitation and growth, and appropriate isolation and identification methods.

9.6.1 Withdrawing Analytical Units

Samples should be analysed as promptly as possible after collection, and if not, stored under conditions that do not permit either death or multiplication of the target microbiota. Paramount in the process of withdrawing sample units is to use aseptic techniques to prevent contamination of the analytical unit. The first step of the preparation for microbiological analysis is to withdraw a representative analytical unit from each sample unit or directly from the pack, whichever is applicable. Samples must be mixed thoroughly to obtain a representative analytical unit. Liquid, semi-liquid and to some extent powders in containers with a headspace, can be mixed by inverting or shaking the container. Analytical units should be removed as soon as possible after mixing. The external parts of packages or containers are first disinfected using most commonly chlorinated, iodinated or alcohol-based solutions. Opening of packages is performed taking all necessary measures to prevent contamination. Where blown packs have been submitted to the laboratory, they should be opened in a biosafety cabinet to avoid danger to the sampler as well as to avoid contamination of the environment. Sterile utensils are used to open packages, mix and withdraw the necessary aliquots.

9.6.2 Dilution and Homogenization of the Analytical Unit

Weighing the analytical unit into the appropriate diluent (first dilution) must be performed aseptically. For products which are easily dispersed in air e.g. powders, it is recommended to work in a microbiological containment cabinet or equivalent to avoid dispersion of dust or aerosols that might be contaminated with pathogens.

Most analytical methods recommend weighing the analytical unit with a precision of ± 0.1 g of the desired weight. The size of the analytical unit has a significant effect on the coefficient of variation of the sample weight. The larger the analytical unit, the lower the coefficient of variation and the better the accuracy. For practical reasons, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, compositing is often used. This is a way of generating a sample that is more typical of a batch than are individual samples tested separately. This is because the ‘between sample’ variance is reduced and the analytical result reflects more closely the true composition of the lot (Jarvis 2007). Compositing comprises taking a number of samples units from different parts of the target sample and combining them into one larger unit that is then subjected to analysis e.g. taking 10 samples of 25 g each and combining them into one sample of 250 g (Corry et al. 2010). When composting for detection of low levels of a particular contaminant, the ability of the testing procedure to detect the contaminant is vital (Jarvis 2007). See Chap. 10 for more information.

For residue collected from the environment, only small quantities, commonly 1 g or less, may be available. Nevertheless, such samples can provide very valuable information. Where a limited quantity of such sample is available, attempts should not be made to ‘bulk up’ to achieve a desired analytical unit quantity as this can lead to a dilution effect.

Some products, such as liquids, semi-liquid and paste-like, are normally mixed and dispersed rather easily. For products such as margarine, butter, chocolate, etc. gentle warming (to about 40 °C) improves dispersion and dissolution of the food matrix and facilitates the release of microorganisms. Other types of samples, in particular solid, require special treatments using homogenizers or blenders.

Diluted homogenized sample units can then be further processed according to the protocols of specific analytical methods. Detailed information on sample dilution and homogenization is provided in different documents such as ISO documents (1999).

Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury that can result from rapid changes in the ionic concentrations. This has long been recognised and is discussed, for example, by Roy (2001), Lee et al. (2006) and Downey et al. (2012).

Dry foods must be rehydrated slowly to avoid die-off due to osmotic shock (e.g., freeze-dried cells) (Ray et al. 1971). This was confirmed by van Schothorst et al. (1979) who showed that the conditions of rehydration of foods can have a major impact on the recovery of salmonellae. Slow rehydration, e.g., using a soaking procedure, resulted in higher recovery rates than shaking.

In foods, the ratio between dead, injured and healthy cells varies, as does the extent of injury to individual cells. Numerous studies have been devoted to this subject and reviews are available (Ray et al. 1971; Mackey 2000).

9.6.3 Recovery of Injured Cells

Some foods exposed to freezing or mild heat treatments or preserved with chemical agents or by low water activity or low pH may contain microorganisms that are sublethally injured. Injury of microbial cells can have a significant impact on analytical results. In such cases, resuscitation conditions are required before these bacteria can be reliably detected by growth in or on selective media. Appropriate resuscitation conditions will allow the injured or damaged cells time to repair that damage and recover all their normal phenotypic and physiological properties. This is necessary before growth is initiated. This "time to repair" is seen as an increased lag-phase and affects the incubation time of both solid and liquid media. Time spans recommended in standard methods are generally those optimal to recover the maximum number of microorganisms. Reducing this incubation period can lead to a significant reduction in counts, or to false negatives in the case of qualitative methods (see Chap. 10). This was shown for *Salmonella*, for example, when pre-enrichment for 6 h gave lower recoveries than pre-enrichment for 24 h. Extending pre-enrichment to 48 h did not increase the numbers of salmonellae and numbers were sometimes lower than those at 24 h (D'Aoust et al. 1992; Hammack et al. 1993).

Food components can markedly affect recovery of both injured and healthy cells, as is shown by detection of *Salmonella* in different food matrices. Rapid rehydration of dehydrated products, such as milk powder or feeds, can reduce recovery rates (Van Schothorst et al. 1979; D'Aoust and Sewell 1986). Dehydrated culinary products, such as soups or concentrated bouillons, and raw materials, such as spices, herbs of onions, require higher dilution rates (1:20 up to 1:100) or the addition of substances to neutralize the inhibitory effects of salt or food components containing naturally occurring antimicrobial components (Andrews et al. 1995). For example, bacteriostatic and/or bactericidal substances present in cocoa may inhibit growth of *Salmonella* during the pre-enrichment (Zapatka et al. 1972). Inhibition is neutralized by adding casein or non-fat dry milk to the pre-enrichment broth (Poelma et al. 1981;). In fatty foods, surfactants such as Tween 80 improve recovery (D'Aoust et al. 1982). Hydrocolloids may affect and inhibit recovery of salmonellae due to thickening and changing the pH of the pre-enrichment broth. Using appropriate dilutions, adding enzymes to reduce viscosity, and adjusting pH have been shown to improve handling and recovery (Amaguaña et al. 1996, 1998). Gel formation of gelatin during incubation will affect recovery, and dilution (1:20) or using papain to reduce the viscosity are recommended (Jay et al. 1997; Amaguaña et al. 1998). Environmental samples can be heavily contaminated and addition of malachite green to the pre-enrichment broth to inhibit competitive microorganisms has been shown to enhance recovery of salmonellae (Van Schothorst and Renaud 1985).

Optimization of pre-enrichment conditions is essential for the detection of salmonellae and should be considered for other pathogens. Increasing the number and type of selective enrichment broths and selective plating media will not improve detection of injured and healthy cells if they are not permitted to recover and grow during pre-enrichment. The recovery of injured cells requires resuscitation to take place on non-selective solid or liquid media, as the selective agents in these media (used to suppress non-target microorganisms) can have an adverse effect on these microorganisms when they are injured (Stephens and Mackey 2012). This is of particular concern if low levels of the target microorganism are present in the test matrix. Selective agents such as sodium lauryl sulphate, Oxgall, brilliant green, bile salts, sodium desoxycholate, crystal violet and others are used in different media to detect Enterobacteriaceae, coliforms or *Escherichia coli*. McCarthy et al. (1998) obtained up to a 3-log reduction in counts of *E. coli* O157:H7 when plated on sorbitol MacConkey Agar in comparison to tryptone soy agar. This has been confirmed by others using different experimental approaches, underlining the need for careful choice of media in the microbiological analysis of foods.

Numerous attempts have been made to overcome this problem through choice of alternate selective agent(s), by introducing a resuscitation step in the procedure, by using a non-selective medium or the addition of betaine, pyruvate or other agents to enhance recovery of injured or stressed cells (Ray 1979; Johnson and Busta 1984; Mackey et al. 1994; Marthi and Lightfoot 1990). See Chap. 10 for more information.

9.7 Discrepancies Between Original and Retest Results

Two very common issues that arise in microbiological testing are:

- One laboratory detects a pathogen in a lot and another laboratory does not.
- Retesting a positive lot fails to confirm the initial result.

When a pathogen is detected in a food product by a two-class attributes plan, there is often a desire to retest retained samples or resample the lot in question to verify the finding. This is particularly true when there are economic and/or public health consequences associated with a positive result.

Often, retesting does not confirm the original positive result even when a much greater portion of product is analyzed in the retest. There may be a desire to believe the original result to be in error, perhaps due to contamination during sampling or analysis. Although laboratory or sampling error are plausible explanations, other explanations also should be considered such as non-random, heterogeneous distribution, low prevalence of contamination, and pathogen die-off between the original and repeat tests.

The discrepancy between original and retest results requires an understanding of how a microorganism may be distributed within a food product, and the difference between prevalence and concentration of contamination. Prevalence refers to the frequency at which multiple samples from a given product test positive. Concentration refers to the number of cells present in a given amount of product. Consider the examples in Fig. 9.1. Both lots, A and B, have the same prevalence but different concentrations. If lots A and B were each divided into 100 one-kg samples and each sample was tested individually with a method capable of detecting one cell kg^{-1}, both lots would likely result in 6 positives per 100 samples tested. However, lot B actually contains a 100-fold higher concentration, because each positive sample contains clumps of approximately 100 cells.

It is common in practice for microbial contamination to exist in clumps as represented in lot B. The number of cells per clump will vary with the nature of the product, source of contamination and stability of the microbial contaminant in the product. Secondly, the distribution of the microorganisms in the product must be considered. If distribution is homogeneous, there is an equal opportunity for contamination to occur at any stage and time of the operation and any individual sample unit would

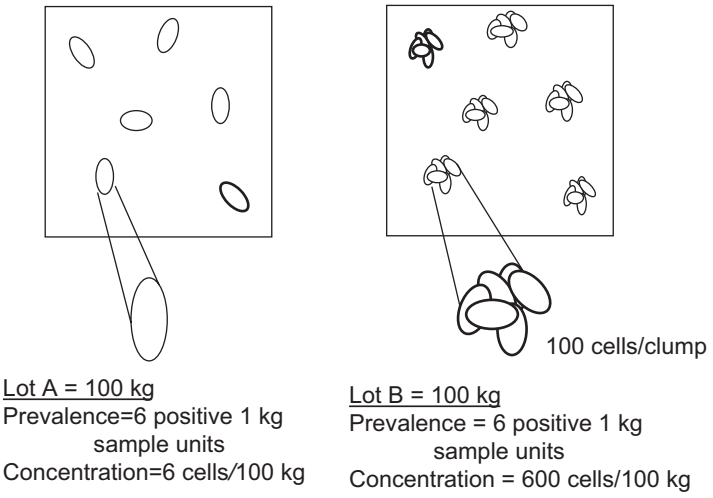
100 cells/clump
Lot A = 100 kg
Prevalence=6 positive 1 kg sample units
Concentration=6 cells/100 kg
Lot B = 100 kg
Prevalence = 6 positive 1 kg sample units
Concentration = 600 cells/100 kg

Fig. 9.1 Illustration of 2 lots of product which upon analysis may yield the same prevalence but have 100-fold different concentrations

be as likely to detect contamination as any other. If, on the other hand, contamination is limited to a certain segment of time during processing, then the defect would not be homogeneously distributed throughout the lot. If heterogeneous, then random sampling may not detect the organism.

Microbiologists commonly refer to random distribution of microorganisms as homogeneous and non-random distribution as heterogeneous. However, this is a misconception, as random refers to an irregular distribution that is still found if the material is "well-homogenized." Figure 9.2a–c illustrate three types of non-random distributions in three lots of 20 consecutively produced boxes. In lot A, the distribution is homogeneous but perfectly regular. Lots B and C represent heterogeneous distributions. In lot B, contamination was greatest in the first sample, followed by decreasing levels of contamination in boxes 2, 3, and 4. This type of distribution of microorganisms commonly occurs when product is produced on contaminated equipment. The product flushes contamination out of the system upon start of production. Lot C is similar to B, but results from contamination being introduced into the system at some time during production. This type of contamination can originate from a variety of sources and causes, e.g., equipment failure followed by substitution of an unclean unit, contamination from line workers or maintenance personnel, contamination introduced from the process environment via aerosol created by cleaning in an adjacent area or contamination from outside the product stream falling into the product.

9.7.1 Non-random Distribution

Most microbiological sampling plans for lot acceptance testing assume contaminants are randomly distributed throughout a lot. In practice, microorganisms are often not randomly distributed, except in mixed liquid samples drawn from a container. Depending on when and how the contamination has occurred, the distribution of contaminants will vary considerably as to location and concentration within a batch. Consider the following examples:

Example 1
Assume, due to a sanitation failure, *Salmonella* has become established in a niche within one of 10 filler heads of a filling machine. During start-up, the first product through this filler head will tend to flush out the contamination so the first product through that filler head will be more highly

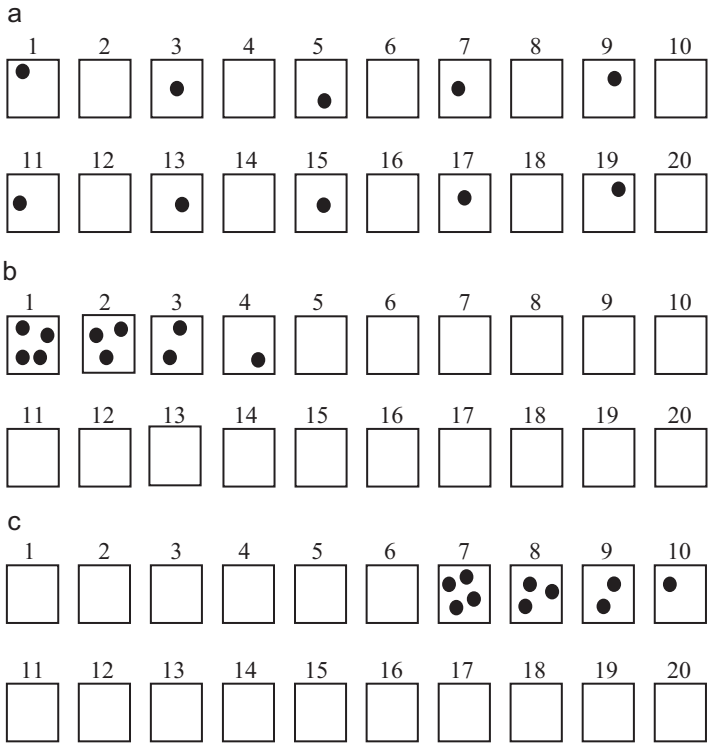

Fig. 9.2 (**a**) Illustration of random distribution throughout a production lot of 20 boxes. (**b**) Illustration of non-random distribution throughout a production lot of 20 boxes. (**c**) Illustration of non-random distribution throughout a production lot of 20 boxes

contaminated. Initially, one in every 10 containers would have *Salmonella*. If samples were collected every few minutes and a quantitative determination performed, the concentration of *Salmonella* in the product from the one filler head would decrease as illustrated as in lot B of Fig. 9.2b.

Analysis of random samples from the lot could by chance detect a positive sample. However, if a retest does not include one or more samples collected from the same filler head early in the production cycle, the initial positive result will not be confirmed even if many samples are analyzed.

Example 2

A combination of condensation and product dust at the top of bucket elevators conveying dry product can provide sufficient moisture and nutrient to allow growth of *Salmonella* and, if introduced, a growth niche can occur during periods of no production. As production resumes, dry product conveyed near the top of the conveyor creates a drier environment. The residue can dry and slough off in clumps into the product stream. In this example, contamination is limited to a particular bucket, with product before or after the event being unaffected. As the product is subsequently transported, perhaps by air or screw conveyor, the clump breaks up and is diluted downstream. The effect is that *Salmonella* within the clump are distributed throughout the product stream much like a comet in space, with the highest level of contamination where the clump entered the product stream, followed by a dilution tail until the stream of product is not contaminated. This is illustrated as Lot C in Fig. 9.2c.

In the above "comet-like" contamination, the first and last packages will be negative; however somewhere within the production lot there is a series of contaminated packages of product. Again, depending on the sampling plan, this contamination may not be detected and if a positive sample is

detected, extensive retesting may not confirm the positive result unless samples are collected from the affected portion of the lot. In practice, "comet-like" contamination can occur with a wide variety of processed foods. For example, one commercial experience involved a dry-blended product that tested positive for *Salmonella* using a sampling plan of $n = 30$. The analytical units (25 g) were composited into two 375 g samples for analysis, for a total of 750 g. All the dry ingredients had been received with certificates indicating they had been sampled and tested negative for *Salmonella*. Since the blending operation was completely dry, there appeared to be little chance for contamination during mixing and packaging. Thus, the ingredients initially were suspected as the source of contamination and the remaining material was extensively resampled.

Eight pallets of a suspect ingredient from an original shipment of 20 pallets remained. Every 25 kg bag was sampled (375 g) and analyzed. Most bags from one pallet of this ingredient and a few bags from another tested positive for *Salmonella*; all others tested negative. Because the bags were sequentially numbered during filling, it was possible to determine that the initial contamination occurred during the middle of the day and decreased to below the detection limit within a couple of hours. Furthermore, one pallet that had been used contained bag numbers within the range of those found positive. The source was further confirmed when the *Salmonella* serotype isolated from the blended product matched the isolate from the ingredient. Obviously, had all three contaminated pallets been used, extensive resampling of the remaining pallets would have yielded negative results and the source of contamination would not have been determined.

Commercial experience with two-class attributes sampling plans for *Salmonella, L. monocytogenes* and other infectious agents clearly demonstrate that, heterogeneous distribution of contamination can be a reason for discrepancies between initial test results and extensive retesting. See Chap. 7 for more information.

9.7.2 Low Prevalence of Contamination in a Lot or Batch

Microbiological criteria specify the quantity of product to be analyzed. Whether a pathogen such as *Salmonella* is detected also depends on its prevalence throughout the lot, its concentration in the analytical unit, and the sensitivity of the analytical method. When present at a high prevalence and concentration sufficient for detection by the test method, the initial analysis and subsequent retests will be positive. When present at a lower prevalence, not every test portion will contain the pathogen. Detection then depends on the probability of including a contaminated (i.e., positive) sample in the analysis. To confirm an initial positive result, a second positive sample must be selected. The probability of selecting a two consecutive positive samples is much lower than the probability of selecting the first positive sample. Confirming an initial positive result when a pathogen is present at low prevalence can be difficult, as described in the following.

In this example, a thoroughly blended product in a 500-L kettle contains one *Salmonella* cell per 3750 g. If a two-class sampling plan that analyses of 375-g sample units, there is approximately one chance in ten that each 375-g test sample will contain one *Salmonella* cell. If the first sample tests positive, then the second 375-g sample will likely be negative. The probability that two samples will test positive in a row is (1 in 10) × (1 in 10) = 1 in 100. Thus, there is only one chance in 100 that two consecutive samples will yield a positive result. Given this situation, a retest used to confirm an initial positive result will almost always increase the risk of accepting a contaminated lot. This is because both the initial and retest samples have equal probability of being positive when contamination is homogeneous. The probability that both will yield a positive result is the square of the probability of a single portion testing positive (Table 9.2). The lower the prevalence of contamination, the more difficult it will be to confirm. Confirmation will depend upon chance or testing until the prevalence of contamination is established. A very low prevalence of contamination is virtually impossible to confirm by resampling.

Table 9.2 Probability of detecting an initial positive and then finding a second positive when the lot is re-tested

Probability of initial positive[a]	Probability of both initial and re-test positive
1 in 2	1 in 4
1 in 5	1 in 25
1 in 10	1 in 100
1 in 20	1 in 400
1 in 50	1 in 2500
1 in 100	1 in 10,000

[a]Probability of a positive = lot size in g × no. cells/size of test sample in g

9.7.3 Low Prevalence of Contamination Across Many Batches

Occasionally, test results indicate that contamination is occurring frequently, but at a very low level. In this example, a 500-L kettle is used to produce 20 lots per month of a homogeneous product and the lots are contaminated with one *Salmonella* cell per 3750 g. Using a sampling plan involving $n = 15 \times 25$-g analytical units to produce a 375-g composite, only two lots will test positive. If sufficient retesting is conducted to establish that the prevalence of contamination in each of the two positive batches is one cell in 3750 g, then it could be concluded that there was one chance in 100 that two positive lots would have been found. Since this is a low probability, one may suspect that the negative lots may also have been contaminated, but at low prevalence. A thorough analysis of several negative lots would determine whether the contamination is widespread. In any event, product histories should be charted and reviewed for sporadic instances of positive test results that indicate low level, widespread contamination (see Chap. 13).

9.7.4 Change in the Concentration of Contamination

In a contaminated ingredient, food, or food processing environment, the number of viable cells may increase, decrease, or remain the same over time. If a pathogen is multiplying, the concentration of contamination (cells g^{-1}) will increase and become easier to detect. However, if death is occurring, the probability of detection will decrease. An example of a survival curve for *Salmonella enterica* introduced onto the surface of spinach leaves as a liquid or absorbed onto poultry manure dust is shown in Fig. 9.3. After 1 day, for the liquid application, with no UV treatment, the number of viable cells decreases rapidly and *Salmonella* reaches the detection limit for plate count. A traditional *Salmonella* test requires approximately 5 days for a positive result. During this time, the concentration of viable cells has decreased to below the detection limit if one continues to use the plate count method. If a retest is requested using the plate count method, without switching to a method with a lower level of detection such as the Most Probable Number (MPN) method, there is an increased probability that the retest will not confirm the initial positive result. Rates for the initial rapid decrease and the subsequent slower decrease (Fig. 9.3) vary by product, pathogen, and storage condition. Extensive experience with preparing inoculated samples for laboratory performance testing, evaluation of new methods, and challenge testing indicates that transferring pathogens such as *Salmonella* from a high moisture growth condition (e.g., broth cultures) into dry product for example, results in a survival curve similar to that shown in Fig. 9.3 for liquid application. A very similar curve would be expected for *Salmonella* that has multiplied in a plant environment and is then introduced into a dry food, for example in many situations where hot/warm air from an open container of product condenses on an over-hanging surface resulting in a growth niche and subsequent ‘spot’ inoculation of dry product. If product is sampled and tested the first few days after production, levels may be

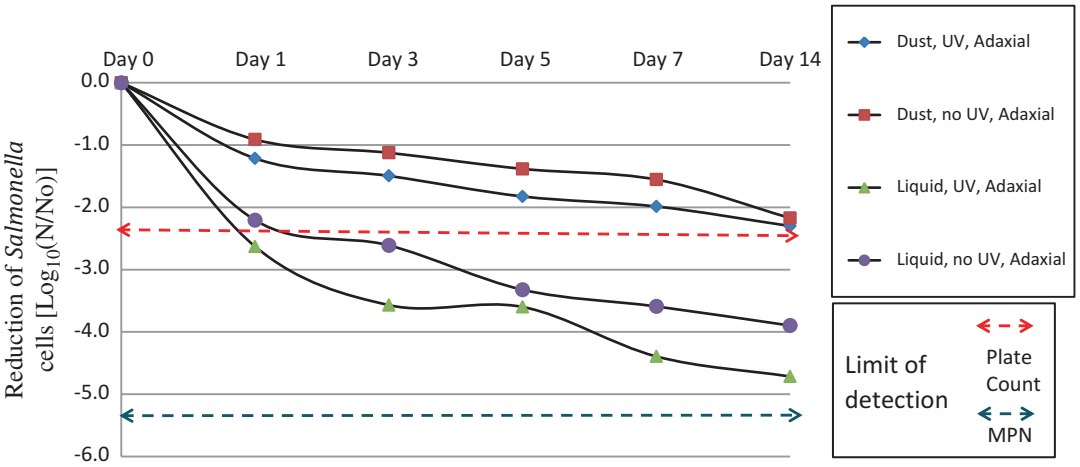

Fig. 9.3 Survival of *Salmonella enterica* on the upper surface of spinach leaves when applied as a liquid or absorbed onto poultry manure dust (Oni et al. 2015)

considerably higher than later when the concentration of survivors is lower, but more stable. The sensitivity of the method is therefore paramount for detecting surviving cells after numbers stabilize and retesting using a less sensitive method may or may not confirm an original positive result.

9.8 Errors Associated with Methods

The errors associated with quantitative methods, such as colony count techniques, differ from those for qualitative methods, such as presence/absence tests (also known as quantal tests). Quality of results is characterized by the accuracy of the method, i.e., the ability to provide results equal to, or close to, the real value. The repeatability (r) of a method reflects the difference between two single results obtained when the same sample is analysed by the same analyst under identical analytical conditions. The reproducibility (R), on the other hand, represents the difference between two laboratories. Examples of r and R-values for different analytes are provided in a number of ISO documents. Errors affecting the quality of data obtained by analytical laboratories have been discussed in detail (Jarvis 2008). Some examples include:

- Laboratory sampling errors, most often characterised by the size of the analytical unit sampled, the homogenous or heterogenous distribution of microorganisms in the primary sample, the accuracy of weighing the analytical unit and differences in handling procedures either in the laboratory or during the sampling process;
- Diluent volume errors which are caused by varying volumes of diluent used in a serial dilution and in the volumes of diluent used in the primary homogenization of samples, which will affect the subsequent dilutions and hence accuracy of the colony counts obtained;
- Pipette volume errors reflected by variations in volumes of liquid dispensed, which are caused by a number of issues including accurate calibration of pipettes and adherence of bacteria to the pipette or pipette tip walls;
- Homogenization and maceration of the food sample which should be conducted in appropriate equipment that allows for adequate homogenization of the sample and does not cause a rise in temperature, thereby avoiding sublethal injury of microbial cells;
- Time lapsing between preparation of dilutions and plating, which should always be consistent and as short as possible;

- Pour plate and related method errors which encompass aspects such as heat-shock of sensitive target microorganisms where poured molten agar is not carefully tempered, inadequate mixing of the agar with the inoculum, spreaders masking the appearance of other colonies, differences in exposure to oxygen, which will affect colony counts;
- Surface plating method errors which include inadequate spreading of the inoculum, inadequate drying of the agar surface, adherence of a portion of the microbial units to the spreading device.

For molecular methods, false positive and false negative results may occur due to a number of factors (Ceuppens et al. 2014). These include:

False positive results:

- Contamination from a positive source, e.g., another food naturally contaminated with the target organism or a positive control used in the laboratory;
- Stringency for the test not being sufficiently high, e.g., non-specific binding of food matrix material with PCR probes or target genes for PCR probe not specific for target organism.

False negative results:

- Too few target sequences present in the volume tested;
- Presence of inhibitory substances (to PCR reaction) in the food matrix;
- Lack of specificity of PCR primers/probes;
- Mutation in the target sequence, resulting in failure of the normal target-probe binding.

9.9 Laboratory Quality Assurance

One of the main goals of the laboratory is the production of high quality analytical data obtained through analytical measurements that are accurate, precise, reliable and adequate for the intended purpose. The ability of a laboratory to consistently achieve high quality analytical data, provides confidence in the laboratory's output. This can be achieved with the implementation of a well established quality assurance program ensuring analytical competency and maintenance of proper documentation. Quality assurance programs require the implementation of elements such as: management quality policy statement, program objectives, control of samples and records, equipment maintenance and calibration of measuring devices, methods evaluation and validation, measurement principles including estimation of uncertainty of measurement, training, methods selection, intra- and inter-laboratory testing, reference standards, field and laboratory sampling, statistical considerations, audits, corrective actions, program revision and update (ISO 2003a, b, c; ISO 2010, 2013; Int'Veld et al. 1995).

9.9.1 Laboratory Accreditation

Laboratory quality management standards such as ISO/IEC 17025 which provide general requirements for the competence of testing and calibration laboratories are available, to which laboratories may seek accreditation by appropriate accreditation organizations. Such organizations provide independent evaluations of compliance of the laboratory to the selected standard and allow the laboratory to demonstrate competency and reliability of their procedures (ISO 2005; FAO 2010). Guidance documents for the implementation of laboratory quality standards have been compiled by a number of organizations to assist laboratories in this quest (ILAC 2002; AOAC 2010).

9.9.2 Validation of Methods

Microbiological methods for testing foods are critical in providing reliable results for a variety of applications, making it important to determine the performance characteristics of a particular method through validation. Validation is the demonstration that a method performs to a set of technical criteria and is an essential part of providing consistently reliable results (AFNOR 2011; FDA 2011b; MicroVal 2013; NordVal 2009). Validation provides confidence to the end user that a method has a proven ability to detect or enumerate the microorganism or group of microorganisms specified (Debevere and Uyttendaele 2000; Jarvis 2008).

Standardized methods have been developed by international, national and trade organizations as well as national governments. Some of these include AOAC International (formerly Association of Official Analytical Chemists) and ISO (International Standards Organization). In food microbiology, validation of both qualitative and quantitative methods generally occurs by comparison to a standardized, classical method; these methods are often used as reference methods.

During the last two decades several alternative, more rapid methods have been developed for detection and / or enumeration of microorganisms in foods. These are often used where a large number of analyses are encountered and where there is a need for rapid results for example for product release or during investigative scenarios. Although such methods play an important role, they require validation to existing reference methods prior to use to ensure their adequate performance for a particular use, i.e., whether the method is fit for purpose. Validation typically includes assessing the method for false positives, false negatives (exclusivity and inclusivity), repeatability and reproducibility. Such assessments include challenging the method with a range of artificially- or naturally-contaminated food matrices to establish whether the method performs as well as its reference counterpart in all of the selected foods against which it is tested. The most commonly used protocols for validating alternative methods have been developed by two international organizations. These are:

- AOAC INTERNATIONAL methods committee guidelines for validation of qualitative and quantitative food microbiological official methods of analysis (Feldsine et al. 2002);
- ISO 16140: Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods (ISO 2003e).

Due to the duplication in the development of these validation protocols, which has resulted in the need for alternative methods being validated against each of the validation protocols, a process has been established to harmonize such protocols.

As demonstrated above, the use of validated methods is critical to ensure the validity and credibility of the data generated. Non-validated methods should not be relied upon for routine analyses and for compliance; however they may be used under certain circumstances, e.g., to provide information or data for advisory purposes.

9.9.3 Good Laboratory Practices and Molecular Techniques

A commonly-used molecular technique to detect microorganisms is the Polymerase Chain Reaction (PCR). The ability of PCR to produce large quantities of copies of a target sequence from minute amounts of DNA has proven to be a powerful diagnostic tool. However, it is important to take the necessary precautions to avoid the generation of false positive results, particularly through laboratory cross contamination. Careful consideration should be given to facility design and operation within laboratories in which polymerase chain reactions are performed. Aspects such as organization of work, specimen processing with physical separation of facilities, equipment and consumables, and other factors should be considered (PHE 2013).

9.9.4 Proficiency Testing

Proficiency testing involves the use of interlaboratory comparisons for the determination of laboratory performance. The need for continued confidence in laboratory performance is essential for laboratories and their customers as well as for other interested parties, e.g., regulators, laboratory accreditation bodies and other organizations that specify requirements for laboratories (ISO 2010). An appropriate international standard exists for proficiency testing i.e. ISO/IEC 17043:2010. It has been developed to provide a consistent basis for all interested parties to determine the competence of organizations that provide proficiency testing. It includes information on typical types of proficiency testing schemes that exist, guidance on the use of appropriate statistical methods and selection and use of proficiency testing schemes by laboratories, accreditation bodies, regulatory bodies and other interested parties (ISO 2010).

Participation of laboratories in proficiency tests organized and offered by national, professional or commercial organisations represents an opportunity for improvements. Such proficiency tests facilitate benchmarking performance of a laboratory by providing laboratories with a means of objectively assessing and demonstrating the reliability of the data they produce. They also aid in identification of weaknesses and can therefore be used as a risk management and performance improvement tool. Where laboratories operate in isolation and do not have ongoing opportunities for comparing their data with others, there are risks that their data may have errors, biases or significant differences from data from other laboratories (ILAC 2011). Proficiency testing can also be applied to assess and improve the performance of analysts (inter-analyst performance) within a laboratory ensuring consistency of analytical data from a laboratory.

It is important to take into account that the type of samples provided for proficiency testing have limitations related to their preparation and the viability of microbial population. Consequently, samples for proficiency testing are not available for all food matrices. The concentration of pathogens included in proficiency test samples is frequently relatively high and competitive microbiota are not always included. Such samples do not, therefore, assess the laboratory's ability to detect very low numbers of injured cells that may occur in actual food samples. The use of reference materials containing very low levels of injured cells, may be more useful in assessing the laboratory performance or reliability of a method. Reference materials have been developed for different microorganisms (Philipp et al. 2007; Abdelmassih et al. 2011).

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Chapter 10
Impact of Sampling Concepts on the Effectiveness of Microbiological Methodologies

10.1 Introduction

As discussed in Chap. 6, there are a number of underlying assumptions that are typically made when developing microbiological sampling plans. For example, it is generally recognized that a basic statistical assumption used for lot evaluations is that the samples are taken randomly to minimize sampling biases. However, there are number of other methodological assumptions that are often made for which there is less understanding in relation to their impact on the effectiveness of microbiological detection. For example, it is commonly assumed in selecting sampling plans that the microbiological methods used are fully effective at recovering the target organism if it is present in a food sample. However, in reality one often has to deal with type I errors (true negatives testing positive) and type II errors (true positives testing negative (AOAC 2006). While great efforts are made to develop and employ methods that meet these methodological assumptions, there are a number of conditions where deviation from these assumptions could have a significant impact on the ability of sample plans to achieve the desired level of confidence. The current chapter will explore several of the methodological factors that could impact the effectiveness of sampling plans, and approaches for quantitatively estimating the decrease or increase in the level of confidence provided by the sampling plan under those circumstances.

A key to determining the impact of methodological factors on the effectiveness of sampling plans is an understanding of the parameters that affect a method's limit of detection (LOD). There are two primary parameters. The first is the inherent number of microorganisms that must be present for the detection signal to be discernable above the "background noise." The second is a result of the particulate nature of microorganisms. When the concentration of the target microorganism is low, detection will be dependent on the probability that the analytical unit actually contains the microorganism. As a simple example, consider a liquid food where the level of a pathogen is 1 CFU/10 ml and the analytical unit plated on a culture medium is 1 ml. If the microbiological method used is 100% effective, then the probability any analytical unit will contain the microorganism is approximately 10%. Data such as this is used to assess the number of "fractional positives" and determine the LOD_{50} for the method (AOAC 2006). At such levels, the limiting factor is the probability that a viable cell is captured in the analytical unit. Thus, within the sample the same concepts of sampling will determine the LOD of the method. How these sampling concepts affect the effectiveness of methodological methods and thus the effectiveness of a sampling plan is discussed in this chapter using a series of examples. The examples consider several methodological factors or conditions to demonstrate how altering the LOD influences the basic assumptions consciously or unconsciously made in the selection of a sample plan.

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10.2 Impact of Microbial Injury on the Effectiveness of Sample Plans

One of the classic methodological problems associated with the microbiological testing of foods is the phenomenon commonly referred to as "injury" (also see Chap. 9). This involves physiological damage to microbial cells as a result of exposure to various stresses (e.g., dehydration, freezing, thermal treatments, exposure to acid conditions, antimicrobial treatments). Generally this is a condition that occurs in conjunction with inactivation of microorganisms. Injury is often a transitional state where the stress being applied has begun to damage the metabolic, physiological, and morphological systems within the cell but not so much as to make the cell non-viable. Ultimately, the stress being applied produces enough cumulative damage that the cell cannot recover and becomes non-viable. Like inactivation, injury often follows first order kinetics, with only a portion of the cells being injured over time (Fig. 10.1). It is important to emphasize that if given appropriate conditions, injured cells can recover, reproduce, and cause disease.

The isolation and identification of microorganisms by cultural means relies heavily on the use of selective and differential microbiological media. Selective plating or enrichment media may employ a variety of chemical agents or conditions for which the microorganism of interest is resistant, thereby permitting its growth while suppressing the growth of other microorganisms. For example, crystal violet is often added to media used for the selective isolation of *Enterobacteriaceae* as a means of suppressing the growth of Gram-positive bacteria. When cells are injured, the metabolic systems damaged often include those that allow the microbial cell to resist other stresses such as toxic elements (e.g., sodium tellurite), antimicrobials (e.g., sorbic acid), antibiotics (e.g., ampicillin), dyes (e.g., crystal violet), emulsifiers (e.g., bile salts), adverse pHs, reduced water activity (e.g., elevated NaCl), and elevated incubation temperatures. The ability of a microorganism to resist one or more of the above agents or conditions is typically the underlying factors that are being used/combined to develop selective and differential media. The loss of resistance due to injury or stress typically results in a corresponding loss of the ability to detect the target microorganism. For example, *Escherichia coli* are characteristically resistant to bile salts, and grow readily in its presence. However when *E. coli* are injured due to exposure to adverse temperatures, pH values, freezing, etc., the injured cells will not grow on bile salts containing microbiological media. Comparing counts on selective and non-

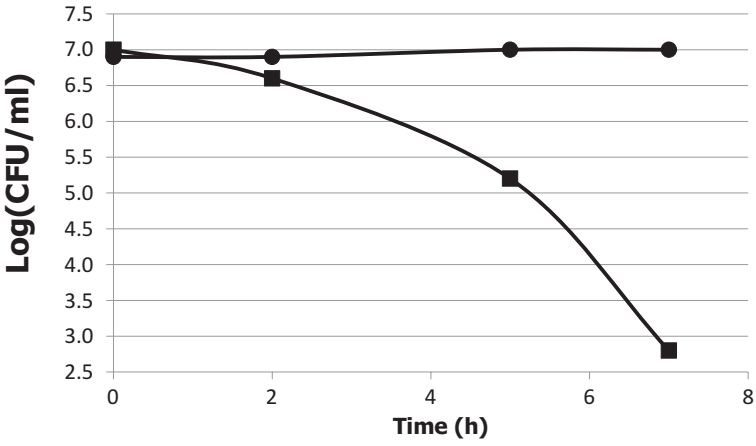

Fig. 10.1 Example of the effect of injury on the quantitative recovery: Recovery of *Escherichia coli* O157:H7 after being exposed to a pH 3.0/83.3 mM acetic acid environment at 37 °C for 7 h (Adapted from Buchanan and Edelson 1999). Selective agar (■), non-selective agar (●)

selective media is the standard method for quantifying the extent of injury in a bacterial population (Chawla et al. 1996). The selective medium provides an estimate of the non-injured cells and the non-selective medium provides an estimate of the total number of viable cells (injured + non-injured).

An immediate implication of the above phenomenon is that microbiological methods that involve the direct use of selective media will no longer fulfill the assumption that if the target microorganism is present in the analytical unit, it will be detected. The result is an effective reduction in the number of detectable cells which will be reflected in a decrease in the number of positive responses in presence/absence tests or the number cells detected in quantitative methods. Since effectiveness of detection is dependent on the concentration of detectable cells, such a reduction could have a significant impact on the effectiveness of a sampling plan.

As a means of exploring this effect in a more quantitative manner, consider a food contaminated with *Salmonella enterica* at a level of 1.0 CFU/g [Log(CFU/g) = 0.00] (Table 10.1). In this example we assume that samples are being directly plated onto a selective medium that does not support the growth of injured *S. enterica* but does support fully the growth of non-injured cells. If 1-g analytical units are being examined and there was no injury (i.e., fulfills the assumption that *S. enterica* in an analytical unit would be detected), the number of samples that would need to be examined to be 95% confident of detecting a contaminated lot is relatively small (i.e., n = 4). However, if the cells are injured, only the non-injured cells will grow, thereby reducing the number of *S. enterica* that will be detected, with the degree of reduction being proportional to the degree of injury. In the current example, the number of replicate aliquots that would need to be tested to ensure 95% confidence increases substantially when the extent of injury is ≥90%.

Potentially the impact of injury could be addressed by making the sampling plan more stringent to a degree proportional to the degree of injury. However, unless the degree of injury was relatively constant (and small), it would require the degree of injury be determined for each sample. While this is theoretically possible, it would make the testing program impractical. Instead, this is the underlying reason why standard protocols for qualitative cultural detection often go to substantial lengths to include pre-enrichment and non-selective enrichment steps that ensure that injured cells have had an opportunity to recover before being subjected to a selective medium. However, a common error in the food microbiology literature is the direct selective enrichment of food samples that are likely to contain a significant percentage of injured cells (e.g., direct selective enrichment of food samples for *Salmonella* without non-selective pre-enrichment).

10.3 Impact of Enrichment Efficiency on the Effectiveness of Sampling Plans

The LOD for most microbiological cultural and non-cultural methods for specific pathogens and indicator organisms is typically greater than the level of public health or regulatory interest, often by several orders of magnitude. For example, most culture methods involving direct plating have a LOD in the range of 10^2–10^3 CFU/g. Likewise, the majority of rapid, genomic-based methods (e.g., real-time PCR) have similar LOD ranges. However, the microbiological criteria for pathogens in specific RTE foods are substantially more stringent. For example, the Codex Alimentarius Commission (CAC) microbiological criterion for *L. monocytogenes* in RTE foods that support growth has a detection goal of 8.0×10^{-3} CFU/g (i.e., presence of 1 CFU in any of five 25-g analytical units [total = 125 g]) and the CAC detection goal for *S. enterica* in powdered infant formula is 6.7×10^{-4} CFU/g (i.e., 1 CFU in any of sixty 25-g analytical units [total = 1500 g]). This represents as much as a 1,000,000–10,000,000-fold differential in detection targets vs. LOD. The standard methodological approach for handling this differential is through the use of one or more enrichment steps. This provides an opportunity for the

Table 10.1 The impact of the degree of injury on the number of samples that would need to be analyzed to ensure a ≥ 95% probability that a lot would be rejected if a liquid food had 1 CFU *Salmonella enterica*/ml (Log(CFU/g = 0.0). The assumptions used in the example are (1) injured *S. enterica* will not grow on the selective medium used to isolate the pathogen, (2) non-injured cells will grow on the selective medium, (3) the analytical unit = 1 ml, (4) the standard deviation is = 0.8, and (5) the assay used is a presence/absence test

True geometric mean concentration of *Salmonella* [Log(CFU/g)]	Percentage of injured cells [%]	Mean concentration of non-injured *Salmonella* [Log(CFU/g)]	Number of samples (*n*) needed to ensure 95% probability of rejection
0.000	0.0	0.000	4
0.000	25.0	−0.125	4
0.000	50.0	−0.301	5
0.000	90.0	−1.000	13
0.000	99.0	−2.000	72
0.000	99.9	−3.000	580

microorganism of interest to attain a sufficiently high level that ensures it is present at levels significantly above the detection method's LOD. The typical approach to enrichment is to prepare composite or pooled samples (also see Chap. 9), combine the composite with an enrichment broth at a ratio of 1:9, incubate at an optimal temperature for 24–48 h, sample the enrichment broth, and assay the sample for the presence of the target microorganism using a selective cultural or non-culture method. If the target microorganism reaches a maximal level of 10^8 to 10^9 CFU/ml, then the level of the microorganism should exceed the detection methods' LOD by at least 100- to 1000-fold. Thus, a basic assumption for microbiological testing that involves an enrichment step is that the target microorganism increases to levels that greatly exceed the detection method's LOD.

Enrichment procedures are generally considered to be the rate limiting step in the detection of microorganisms in foods. Accordingly, there has been a great deal of interest and research into accelerating the enrichment step by decreasing the size of the analytical units being composited/pooled and enriched, increasing the number of analytical units being composited, shortening the time of enrichment, decreasing the extent of the initial dilution (e.g., 1:2 sample-to-medium ratio instead of 1:9), decreasing the selectivity of the enrichment to avoid problems with injured cells, or decreasing the size of the aliquots from the completed enrichment that are subsequently tested for the target microorganism. Potentially each of these factors can affect the final concentration of the target microorganism being analyzed, which in turn could impact the effectiveness of the selected sampling plan. As a means of demonstrating the impact that some of these methodological factors could have on the efficacy of sampling plans, three examples will be considered: (1) the effect of the extent of growth in the enrichment broth, (2), the volume of broth from the completed enrichment examined for the target microorganism, and (3) the effect of the duration of incubation and rate of growth of the target microorganism.

10.3.1 Effect of Extent of Growth in Enrichment Broth

As mentioned above, a key methodological assumption for methods that include an enrichment step is that the target microorganism attains a level that substantially exceeds the detection method's LOD. However, there are instances where this may not be the case. As we will explore more fully below, the presence of an inhibitory compound or condition, a competing microbiota, a significant level of injured cells or shortened incubation duration can result in a depressed level of growth during the enrichment period. As a means exploring these effects, we will consider a simple hypothetical

Table 10.2 A hypothetical example of the impact of levels of enterohemorrhagic *E. coli* O157:H7 after enrichment on the subsequent detection of the microorganism. The example assumes that 50-µl aliquots of the incubated enrichment broth are plated on one, three, or five Sorbitol-MacConkey plates, incubated, and then examined for the presence/absence of *E. coli* colonies[a]

Mean concentration of *E. coli* after enrichment [Log(CFU/ml)][b]	Probably of accepting (P_a = %) a lot initially contaminated with a low level of *E. coli*		
	n = 1	n = 3	n = 5
−2.0	99.9	99.8	99.7
−1.0	99.5	98.4	97.3
0.0	94.6	84.7	75.9
+1.0	59.2	20.8	7.9
+2.0	2.2	0.0	0.0
+3.0	0.0	0.0	0.0

[a]It is assumed that if an *E. coli* cell is present in a 50-µl aliquot, it will be detected
[b]A standard deviation of 0.2 is assumed based on the homogeneous nature of the enrichment process

example where a composited food sample containing low levels of enterohemorrhagic *E. coli* O157:H7 is enriched for 24 h. After incubation, one, three, or five 50-µl (0.05 ml) portions of the enrichment broth are plated onto Sorbitol MacConkey Agar. After incubation, the plates are inspected for the presence/absence of colonies characteristic of sorbitol-negative *E. coli*. The effect of the target microorganism reaching final mean concentrations of 10^{-1} to 10^{3} CFU/ml in the enrichment broth on the likelihood of detecting *E. coli* after plating on one, three, or five samples is depicted in Table 10.2. A standard deviation of 0.2 was assumed since this is a well-mixed liquid system that should have minimal variability. It is apparent in this example that if the level of *E. coli* O157:H7 in the enrichment broth did not achieve 10^{2} CFU/ml or higher there is an increasing probability that the lot would be accepted despite the presence of low levels of the pathogen. This emphasizes the need to ensure that either the enrichment step achieves sufficient growth or modify the sampling plan to match the capability of the enrichment system. Generally the focus of most standard methods is to develop enrichment systems that achieve a high degree of growth.

10.3.2 Effect of the Volume of Broth from Completed Enrichment Examined for Target Microorganism

As introduced above, there has been an increased trend on the part of developers of microbiological detection systems to decrease the size of analytical units. This includes both (a) reducing the size of analytical units being examined either through direct enrichment of individual or composited samples or (b) reducing the volume of the enrichment broth that is subsequently analyzed by either a cultural or non-cultural detection system. The impact of the former has been discussed in Chap. 6 and will not be covered here. The impact of the volume of the enrichment broth analyzed reflects that probability that a cell of the target microorganism is actually present in the portion of the enrichment being examined. As a means of demonstrating the effect of volume analyzed, let's return to the example from Sect. 10.3.1, the detection of enterohemorrhagic *E. coli* O157:H7 on Sorbitol-MacConkey Agar. In this case we will assume that during enrichment the microorganism attains a mean concentration of 10^{2} CFU/ml in the enrichment broth. Based on 2-class presence/absence testing statistics, the probability of detecting a single colony as a function of the volume of enrichment broth plated (volume = 1, 2, 5, 10, and 50 µl) was calculated (Table 10.3) for n = 1, n = 3, and n = 5.

Table 10.3 A hypothetical example of the impact of sample size of a completed enrichment of a food sample containing low levels enterohemorrhagic *E. coli* O157:H7 on its subsequent detection. The example assumes that the microorganism attains a mean concentration in the enrichment broth of 10^2 CFU/ml. After enrichment, the example assumes that 1, 2, 5, 10, or 50-µl aliquots of the completed enrichment broth are plated on one, three, or five Sorbitol-MacConkey Agar plates, incubated, and then examined for the presence/absence of sorbitol-negative *E. coli* colonies[a]

Volume of enrichment broth plated on Sorbitol-MacConkey Agar (µl)	Probably of accepting (P_a = %) a lot initially contaminated with a low level of *E. coli* O157:H7		
	n = 1	n = 3	n = 5
50	2.18	0.00	0.00
10	36.9	5.04	0.69
5	59.3	20.8	7.3
2	80.5	52.2	33.8
1	89.6	71.9	57.8

[a]It is assumed that if an *E. coli* cell is present in the enrichment broth aliquot, it will be detected
[b]A standard deviation of 0.2 is assumed based on the homogeneous nature of the enrichment process

The transfer volumes selected were intended to cover the ranges currently used in conjunction with a number of cultural and non-cultural detection systems. For the non-cultural methods based on the detection of DNA, it is assumed that each cell contains one copy of a chromosomal target gene, and can be treated statistically in a manner similar to that for intact cells. For genomic techniques where there are multiple copies per cell (e.g., detection of ribosomal nucleic acids or multiple insertion elements), this assumption would need to be modified, and helps explain an advantage that such methods may have in terms of LODs.

It is apparent that the volume examined can have a substantial impact on the probability of detecting the microorganism, particularly if the level of *E. coli* attained is relatively low. In this example, the probability of accepting a lot of food that contained low levels of *E. coli* O157:H7 increased substantially as the volume of enrichment broth analyzed (and thus the number of cells captured) decreased. The impact of volume of enrichment analyzed is strongly dependent on the level that the microorganism attains during enrichment (see Sect. 10.3.1). Potentially, the sampling plan could be modified to overcome the problem of low levels of the target organism after enrichment by increasing the volume of the analytical units and/or number of analytical units examined. However, the primary approach to avoiding this problem is to again develop enrichment systems/protocols that support growth of the target microorganism to high levels and to analyze as large an aliquot of the enrichment broth as practical.

10.3.3 Effects of Incubation Time and Target Microorganism Growth Rate

As noted in the two examples above, the key to successful enrichment protocols is to ensure that the target microorganism grows rapidly and reaches high concentrations within the designated incubation period. Despite attempts to design universal enrichment protocols to ensure this happens, there are instances where this growth of the target microorganism may become non-optimal. For example, if transfer of a highly acidic food sample to an enrichment broth overcomes the broth's buffering capacity, the reduced pH could slow the growth of the target microorganism. The use of a selective enrichment system will likely lead to either an effective reduction in the concentration of the target organism in the enrichment broth due to lack of growth by injured cells (see Sect. 10.2) or delay the growth of the target microorganism due to an extended lag phase or the time needed for recovery of injured cells. Similarly, the presence of significant levels of a bacteriostatic antimicrobial in the food could

lead to insufficient growth in the enrichment culture. This becomes particularly important if the duration of the enrichment incubation is shortened. As a means of demonstrating the impact of growth rate and enrichment broth conditions on the effectiveness of sampling plans, two hypothetical examples involving the detection of *L. monocytogenes* from an enrichment broth will be considered.

The first example considers the effect of enrichment broth incubation times and pH on the likelihood that *L. monocytogenes* present at an initial concentration of 1 CFU/100 g will be detected when 50 µl of the completed enrichment broth is plated onto PALCAM Agar. As in prior examples, the probability of detection will be based on the presence/absence of colonies indicative of *L. monocytogenes* on the PALCAM Agar plates. As before, it is assumed that the enrichment protocol involves mixing the food and enrichment broth at a ratio of 1:9. To decrease the potential that we enriched a sample of food that did not contain at least one *L. monocytogenes* cell, we will assume that a 300-g composited sample is combined with 2700 ml of enrichment broth, i.e., the initial concentration of *L. monocytogenes* in the enrichment broth is approximately 1 CFU/1000 ml [Log (CFU/ml) = −3.0]. Growth of the target microorganism was calculated using the aerobic model for *Listeria monocytogenes* from the USDA Pathogen Modeling Program to predict lag phase duration and generation times (Table 10.4). The levels predicted in the enrichment broth were then used to calculate the probability that the lot from which the sample was drawn would be accepted as not containing *L. monocytogenes* (Table 10.5).

The results clearly demonstrate the probability of detecting a contaminated lot is dependent on the duration of the incubation period and the pH of the enrichment broth. An incubation period of 18 h provides a high degree of confidence that the original contamination levels would be detected, particularly if multiple analytical units (n = 3 or n = 5) were examined. However, this would not be sufficient if the pH of the enrichment broth was pH 5.0. The transition from poor to excellent detection probabilities is relatively abrupt, particularly under conditions that support rapid growth. This reflects the shortened lag times and the exponential nature of microbial growth. This example provides a quantitative example of the dependency of sampling plans that involve enrichment protocols on sufficient growth of the target microorganism.

The second example extends the one just discussed by considering the impact of injury. In this scenario let's assume that a highly mixed food product (e.g., milk) receives a mild heat treatment that is insufficient to inactivate *L. monocytogenes* but is sufficient to injure 99.99% of the cells. In this example we will assume that the injured cells' lag phase is extended by 4 h as they undergo recovery prior to growth in a selective enrichment medium. We will assume further that the concentration of *L. monocytogenes* in food is 10^0 CFU/g. The method of analysis involves adding 10 g of the food to 1, 3, or 5 flasks containing 90 ml of enrichment broth. As in the prior example, the effect of pH and incubation temperature of the enrichment broth is considered. Thus, the effective concentration of *L. monocytogenes* in the enrichment broth is Log (CFU/ml) = −1.0 for total cells (injured + non-injured) and Log (CFU/ml) = −4.0 for non-injured cells. Since the probability of getting a non-injured cell in an enrichment tube is small, the growth of non-injured cells will be ignored in the example. The flasks are incubated at 25 and 37 °C and then 50 µl aliquots of each flask is plated onto PALCAM Agar similar to the previous example (Table 10.5) and examined for the presence of at least one *L. monocytogenes*

Table 10.4 The growth kinetics predicted by the USDA Pathogen Modeling Program for the growth of *Listeria monocytogenes* in an enrichment broth having different pH values and incubated at 25 °C or 37 °C[a]

Growth kinetics	Incubation temperature [°C]	pH 5.0	pH 6.0	pH 7.0
Generation time (h)	25	1.7	0.7	0.6
Lag time (h)		14.1	4.5	3.8
Generation time (h)	37	0.8	0.4	0.3
Lag time (h)		5.6	1.9	1.7

[a]Assumed that the medium contained 0.5% NaCl and 0.0 ppm $NaNO_2$

Table 10.5 A hypothetical example of the effect of enrichment incubation time, incubation temperature, and pH on the probability that a food sample containing low levels of *Listeria monocytogenes* would be detected by subsequent plating on PALCAM Agar[a]

Incubation time (h)	Incubation temperature [°C]	Probability (%) that a lot would be accepted based of the results of the enrichment[b,c]											
		pH 5.0				pH 6.0				pH 7.0			
		Predicted Log(CFU/ml)	n = 1	n = 3	n = 5	Predicted Log(CFU/ml)	n = 1	n = 3	n = 5	Predicted Log(CFU/ml)	n = 1	n = 3	n = 5
3	25 °C	−3.00	99.99	99.98	99.97	−3.00	99.99	99.98	99.97	−3.00	99.99	99.98	99.97
6		−3.00	99.99	99.98	99.97	−2.35	99.98	99.93	99.88	−1.90	99.93	99.73	99.65
12		−3.00	99.99	99.98	99.97	0.23	91.08	75.57	62.69	1.12	50.72	13.05	3.36
18		−2.31	99.97	99.92	99.86	2.81	0.00	0.00	0.00	4.13	0.00	0.00	0.00
24		−1.25	99.69	99.07	98.45	5.39	0.00	0.00	0.00	>7.00	0.00	0.00	0.00
3	37 °C	−3.00	99.99	99.98	99.97	−2.17	99.96	99.89	99.81	−1.69	99.89	99.66	99.43
6		−2.84	99.99	99.98	99.97	0.09	93.44	81.58	71.23	1.32	35.49	4.47	0.56
12		−0.59	98.58	95.81	93.12	4.61	0.00	0.00	0.00	>7.0	0.00	0.00	0.00
18		1.67	12.37	0.19	0.00	>7.0	0.00	0.00	0.00	>7.0	0.00	0.00	0.00
24		3.93	0.00	0.00	0.00	>7.0	0.00	0.00	0.00	>7.0	0.00	0.00	0.00

[a]The example assumes that one, three, or five 50-µl portions of the enrichment broth plated on PALCAM Agar plates at the designated incubation times. A standard deviation of 0.2 is assumed due to the well mixed nature of the enrichment broth

[b]Initial concentration in enrichment broth was assumed to be −3.0 Log(CFU/ml) (see text). The growth kinetics (generation time and lag phase duration) of *Listeria monocytogenes* in the enrichment broth were calculated using the aerobic model from the USDA Pathogen Modeling Program with the sodium nitrite parameter being set to 0.00 and the NaCl levels assumed to be 0.5%

[c]Probability of acceptance (P_a) values were calculated using ICMSF model for presence/absence testing using a 2-class attribute plan

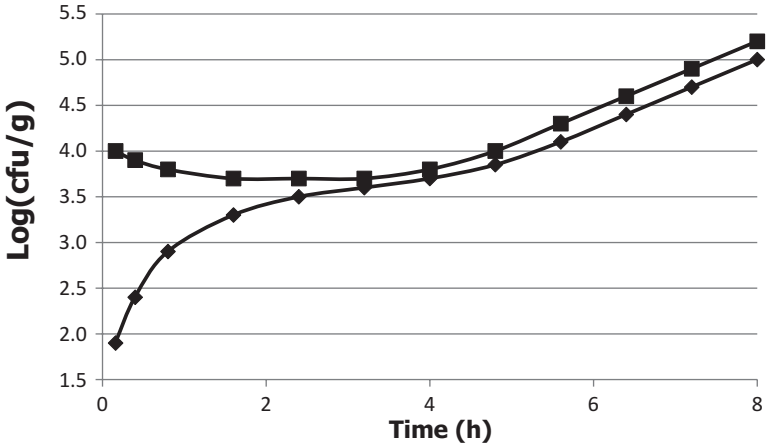

Fig. 10.2 Typical recovery of injured cells as evidenced by comparative growth on selective and non-selective media. Selective medium (●), non-selective medium (■)

colony of the plate. The growth parameters used are the same as those in the previous example (Table 10.4). For the purpose of comparison, a second set of enrichments for food that has not been injured is considered.

In developing this example, it was necessary to consider the behavior of injured cells during recovery (Fig. 10.2). For the sake of simplicity, it is assumed that the recovery period is independent of the lag phase, i.e., it is added onto the lag phase and is independent of the pH or incubation temperature of the enrichment broth. After the recovery period, the growth kinetics values are the same as those for non-injured cells. Based on this assumption, the effect of injury on the probability of lot acceptance is provided in Table 10.6.

As in the prior example, the potential detection of *L. monocytogenes* was dramatically influenced by the duration of the enrichment period if such a step is required to achieve a high likelihood that the microorganism is present in the ultimate analytical unit used for detection. This is further influenced by the ability of the enrichment conditions to support maximal growth. Injury and other sub-lethal stresses further affect the process by effectively extending the lag phase of the injured cells. As can be seen in this example, injury extended the required enrichment process, pointing out the challenges of trying to reduce the current recommended enrichment incubation periods. As previously discussed, increasing the stringency of the intra-method sampling (e.g., number of replicate enrichments), the emphasis on developing effective enrichment media and protocols is the primary focus for avoiding what could be a substantial source of type 2 errors.

10.4 Impact of Microbial Competition and Mixed Microbiota on Effectiveness of Detection Methods and Ultimately Sampling Plans

Often one of the unstated assumptions in the testing of foods for specific microorganisms is that the target microorganism behaves independently, i.e., the growth of the target microorganism during enrichment is unaffected by the presence other microorganisms. This assumption generally holds true when dealing with low levels of both the target microorganism and other microbial entities. However, this assumption may not be valid when dealing with high concentrations of other microorganisms such

Table 10.6 A hypothetical example of the effect of injury, enrichment incubation duration, and pH of the enrichment broth on the probability of acceptance of food that has a low level of *Listeria monocytogenes*[a]

Incubation time (h)	Incubation temperature [°C]	Injury Status[d]	Probability (%) that a lot would be accepted based of the results of the enrichment[b,c]											
			pH 5.0				pH 6.0				pH 7.0			
			Predicted Log(CFU/ml)	n = 1	n = 3	n = 5	Predicted Log(CFU/ml)	n = 1	n = 3	n = 5	Predicted Log(CFU/ml)	n = 1	n = 3	n = 5
3	25	I	−1.00	99.45	98.35	97.26	−1.00	99.45	98.35	97.26	−1.00	99.45	98.35	97.26
		N	−1.00	99.45	98.35	97.26	−1.00	99.45	98.35	97.26	−1.00	99.45	98.35	97.26
6		I	−1.00	99.45	98.35	97.26	−1.00	99.45	98.35	97.26	−1.00	99.45	98.35	97.26
		N	−1.00	99.45	98.35	97.26	−0.35	97.55	92.84	88.36	0.10	93.29	81.20	70.67
12		I	−1.00	99.45	98.35	97.26	0.51	83.84	58.94	41.43	1.11	51.46	13.62	3.61
		N	−1.00	99.45	98.35	97.26	2.22	0.40	0.00	0.00	3.11	0.00	0.00	0.00
18		I	−1.00	99.45	98.35	97.26	3.08	0.00	0.00	0.00	4.12	0.00	0.00	0.00
		N	−0.31	97.32	92.18	87.31	4.81	0.00	0.00	0.00	6.13	0.00	0.00	0.00
24		I	0.05	94.00	83.05	73.37	5.67	0.00	0.00	0.00	>7.00	0.00	0.00	0.00
		N	0.75	73.94	40.42	22.10	>7.00	0.00	0.00	0.00	>7.00	0.00	0.00	0.00
3	37	I	−1.00	99.45	98.35	97.26	−1.00	99.45	98.35	97.26	−1.00	99.45	98.35	97.26
		N	−1.00	99.45	98.35	97.26	−0.17	96.33	89.38	82.94	0.31	89.40	71.46	57.11
6		I	−1.00	99.45	98.35	97.26	−0.92	99.33	98.02	96.72	0.31	89.40	71.46	57.11
		N	−0.85	99.22	97.67	96.15	2.09	1.15	0.00	0.00	3.31	0.00	0.00	0.00
12		I	−0.10	95.70	87.65	80.28	3.59	0.00	0.00	0.00	6.33	0.00	0.00	0.00
		N	1.41	28.73	2.37	0.20	6.60	0.00	0.00	0.00	>7.00	0.00	0.00	0.00
18		I	2.16	0.66	0.00	0.00	>7.00	0.00	0.00	0.00	>7.00	0.00	0.00	0.00
		N	3.67	0.00	0.00	0.00	>7.00	0.00	0.00	0.00	>7.00	0.00	0.00	0.00
24		I	4.42	0.00	0.00	0.00	>7.00	0.00	0.00	0.00	>7.00	0.00	0.00	0.00
		N	5.93	0.00	0.00	0.00	>7.00	0.00	0.00	0.00	>7.00	0.00	0.00	0.00

[a]The example assumes that one, three, or five 50-µl portions of the enrichment broth plated on PALCAM Agar plates at the designated incubation times. A standard deviation of 0.2 is assumed due to the well mixed nature of the enrichment broth

[b]Initial concentration of *L. monocytogenes* in the food as assumed to be Log(CFU/g) = 0.0, and the initial concentration in enrichment broth was Log(CFU/ml) = −1.0 (see text). The growth kinetics (generation time and lag phase duration) of *Listeria monocytogenes* in the enrichment broth were calculated using the aerobic model from the USDA Pathogen Modeling Program with the sodium nitrite parameter being set to 0.00 and the NaCl levels assumed to be 0.5% (see Table 10.4)

[c]Probability of acceptance (P_a) values were calculated using ICMSF model for presence/absence testing using a 2-class attribute plan

[d]I = injured, N = non-injured. In the case of injured cells, the food was subjected to a treatment that injured 99.99% of the cells without lethality, and that their recovery required 4 h (see text)

as conditions that might be encountered during an enrichment step with foods that have a substantial microbial burden, either naturally occurring (e.g. fermented foods) or deliberately added (e.g. probiotic-containing products). This would be particularly true in cases where non-selective enrichment is employed. Potentially, two different problems could arise. The first is when the growth of another microorganism interferes with the growth of the target microorganism. The second is when the growth of a closely related microorganism interferes with the detection of the target microorganism. Examples for each of those situations will be considered below.

10.4.1 The Potential Impact of the Jameson Effect on the Effectiveness of Sampling Plans

One of the characteristics of microbiological systems where there are two of more competing microorganisms is that if one of the microorganisms has a significantly faster growth rate it will suppress the maximum level attained by the second microorganism. The phenomenon is referred as the Jameson Effect. Typically, as the dominate microorganism enters early stationary phase (e.g., 10^8 to 10^9 CFU/g), the secondary microorganism ceases growth and enters stationary phase at a level that can be substantially reduced from what would be expected if it was growing alone. An example of the Jameson Effect for *L. monocytogenes* growing in the presence or absence of *Pseudomonas fluorescens* is provided in Fig. 10.3. Various explanations for the effect have been hypothesized such as depletion of micronutrients, the generation of inhibitory compounds (e.g., bacteriocins, chelating agents), or alternation of the environments (e.g., reduction in pH). The suppression of *L. monocytogenes* growth during enrichment due to the growth of competing microorganisms has been observed with a number of *Enterobacteriaceae* (Dailey et al. 2014). Three of the factors that may influence the extent of a Jameson Effect are the relative growth rates and lag phase durations of the two microorganisms, the relative ratio of the initial levels of the two organisms, and the ability of the predominate organism to produce inhibitory agents that affect the target organism.

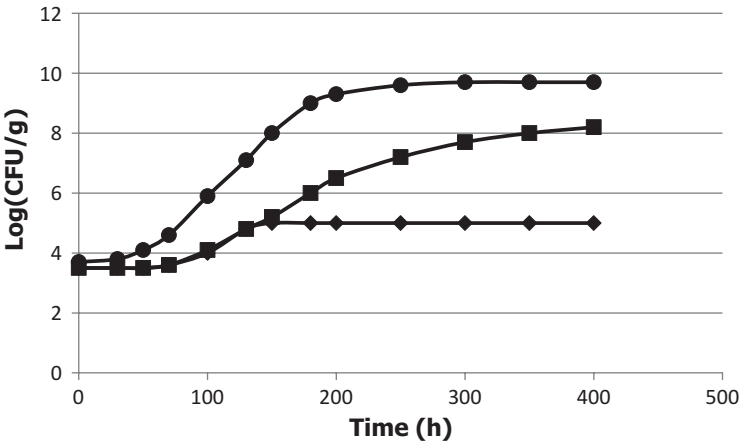

Fig. 10.3 Example of the Jameson Effect: Suppression of the growth of *Listeria monocytogenes* in the presense of *Pseudomonas fluorescens* when incubated together at 4 °C In Brain Heart adjusted to pH 6.0 (Adapted from Buchanan and Bagi 1999). *P. fluorescens* (●), *L. monocytogenes* in the presence of *P. fluorescens* (◆), *L. monocytogenes* in the absence of *P. fluorescens* (■)

Table 10.7 Example of the potential impact of the Jameson Effect on the enrichment of low levels of *Listeria monocytogenes*[a]

Initial level of competing microorganism [Log(CFU/ml)]	Time for the competing microorganism to achieve population density of 10^9 CFU/ml [h]	Levels of *Listeria monocytogenes* attained when competitor reaches 10^9 CFU/ml [Log(CFU/ml)]
1.0	10.0	3.0
2.0	8.8	2.4
3.0	7.5	1.8
4.0	6.3	1.2
5.0	5.0	0.5
6.0	3.8	−0.1
7.0	2.5	−0.8

[a]Assumed that when a competing microorganism achieves a population density of 10^9 CFU/ml that stops the growth *L. monocytogenes*. It is further assumed that exponential growth rate of *L. monocytogenes* is 0.5 log cycles/h while the rate for the competitor is 0.8 log cycles/h. It is also assumed that both microorganisms are growing exponentially, and the initial level of *L. monocytogenes* in the enrichment broth is Log(CFU/ml) = −2.0

As an example of the potential impact that the Jameson Effect can have on the effectiveness of sampling plans and their accompanying microbiological methods, a simple example of competition during the enrichment phase of a method for the detection of low levels of *L. monocytogenes* from foods. In this example it is assumed that there is a second commonly occurring microorganism that stops the growth of *L. monocytogenes* when the competitor reaches 10^9 CFU/ml in a manner similar to that depicted in Fig. 10.3. In this example, we will assume that the exponential growth rate of *L. monocytogenes* is 0.5 log cycles/h while the exponential growth rate for the competitor is 0.8 log cycles/h. As a way of simplifying the example, it is assumed that both microorganisms are out of the lag phase and growing exponentially. The final assumption is that the initial level of *L. monocytogenes* in the enrichment broth is Log(CFU/ml) = −2.0. After incubation of the enrichment culture, one, three, or five 20 µl aliquots of the enrichment broth are spread plated on PALCAM Agar, incubated and examined for representative colonies.

The levels of *L. monocytogenes* attained in the enrichment broth are dependent on the initial level of the competing microorganism (Table 10.7). In this example, when the initial ratio of competitor to *L. monocytogenes* is 1000:1, the pathogen is able to increase 100,000-fold before the competitor exerts a Jameson Effect. However, when the competitor is present at higher concentrations that might be expected with many raw commodities, the replication of *L. monocytogenes* is severely impacted. When the likelihood that *L. monocytogenes* would be detected when one, three, or five 20-µl analytical units are then plated on a selective/differential agar, substantial differences in the probability of acceptance of a lot are observable (Table 10.8). It is readily apparent that the assumption that the enrichment step provides a high degree of confidence that level of *L. monocytogenes* greatly exceeds the LOD is not met in this example, if the level of the competing microorganism was elevated. Based on this hypothetical example, the level of the competitor would need to be less than approximately 100 CFU/ml if a single 20 µl to analytical unit from the enrichment broth was being examined to determine the presence of *L. monocytogenes*.

10.4.2 Effect of Differential Growth Rates on the Confirmation of the Identity of Pathogenic Species or Strains

As demonstrated above, the detection of low levels of pathogenic microorganisms from foods typically requires the inclusion of an enrichment step in order to increase the numbers sufficiently to ensure that the LOD of the method is exceeded and there is a high probability that the target

Table 10.8 Example of the potential impact of the Jameson Effect on the recovery of low levels of *Listeria monocytogenes*

Level of *L. monocytogenes* attained after enrichment in the presence of a competing microorganism[a] [Log(CFU/ml)]	Probability (%) that a lot would be accepted based of the results of the enrichment[b,c]		
	n = 1	n = 3	n = 5
3.0	0.01	0.00	0.00
2.4	2.15	0.00	0.00
1.8	29.31	2.52	0.22
1.2	71.25	36.17	18.37
0.5	93.26	81.12	70.56
−0.1	98.25	94.85	91.56
−0.8	99.65	98.95	98.25

[a]See Table 10.7 and text

[b]Probability of acceptance (P_a) values were calculated using ICMSF model for presence/absence testing using a 2-class attribute plan. Standard deviation of 0.2 was assumed

[c]The example assumes that one, three, or five 20-μl portions of the enrichment broth were plated on PALCAM Agar plates

microorganism is captured within an analytical unit. In many methods, the detection of the target microorganism from the enrichment broth involves plating on differential or differential/selective plating medium and then picking representative colonies to confirm the identity of the isolate. However, this protocol can result in significant type 2 errors if there is a second microorganism that grows on the plating medium that cannot be easily distinguished from the target microorganism. This can be a particular problem if the second microorganism has a faster growth rate or a significantly greater concentration in the initial enrichment broth than the target pathogen. For example, it has been long recognized that the presence of *Listeria innocua* in a food can interfere with the accurate detection of *L. monocytogenes* (Petran and Swanson 1993; Curiale and Lewus 1994; MacDonald and Sutherland 1994; Cornu et al. 2002) and will be used as a hypothetical example of the difference in growth rates impacting the effectiveness of determining the presence of the pathogen.

In this example we will assume that a homogeneously mixed food contains *L. monocytogenes* at a level of 1 CFU/10 g and *L. innocua* at 1 CFU/100 g, 1 CFU/10 g, or 1 CFU/g. The food sample is mixed at a ratio of 1:9 with an appropriate enrichment broth, so that the initial concentration of *L. monocytogenes* in the enrichment broth is Log (CFU/ml) = −2.0 and the initial concentrations of *L. innocua* are Log(CFU/ml) = −3.0, −2.0, or −1.0. The enrichments are then incubated at 25 °C for 24 h, and a 1.0 ml aliquot of the enrichment broth was diluted appropriately so that when spread plated on PALCAM agar there were approximately 100–300 colonies per plate. Colonies (1, 5, 10, or 20) were selected and tested to determine whether they were *L. monocytogenes* or *L. innocua*. This lot would be accepted if none of the colonies selected were *L. monocytogenes* with n equal to the number of colonies examined and c = 0.

It is assumed in this example that the lag phase duration and generation time were 3.0 h and 1.0 h for *L. monocytogenes*, and 2.5 h and 0.8 h for *L. innocua*. The predicted growth of *L. monocytogenes* and *L. innocua* are depicted in Fig. 10.4.The probability of acceptance is calculated using a hypergeometric distribution (Jarvis 2008):

$$P_{(x=0)} = \left(\alpha!\left(N-n\right)!/\left(\alpha-n\right)!N!\right)$$

where N = number of colonies on the plate, n = number of colonies picked for identification, and α = number of non-target microorganisms on the plate.

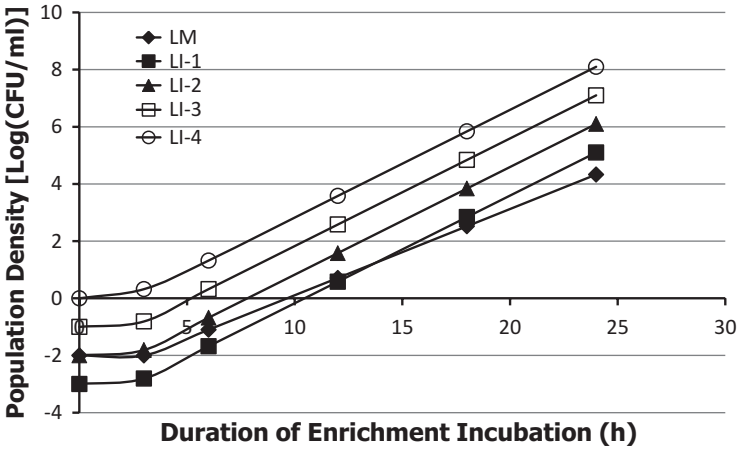

Fig. 10.4 Predicted growth of *Listeria monocytogenes* (LM) and four levels of *Listeria innocua* (LI 1–4) during enrichment at 25 °C

The probability of picking a *L. monocytogenes* on a PALCAM agar plate for each of the initial *L. monocytogenes* / *L. innocua* concentrations is summarized in Table 10.9. In this hypothetical example, if the final concentrations of the two microorganisms is approximately the same at the end of enrichment, there is a high likelihood that a least one *L. monocytogenes* would be detected, particularly if at least five colonies are examined. However, as the ratio of *L. innocua* to *L. monocytogenes* increases, the relative presence of the *L. monocytogenes* drops precipitously and the probability of detecting a *L. monocytogenes* colony becomes disappearingly small. This could be overcome to some degree by increasing the number of colonies examined; however, this would have limited utility due to the practicality of examining such a large number of colonies. Instead, the better solution would be to develop either an enrichment medium that discourages the growth of *L. innocua* during enrichment or a plating medium that allows direct differentiation of the two microorganisms.

10.5 Impact of Immunomagnetic Concentration Systems on the Effectiveness of Sampling Plans

It is apparent from the different examples provided in this chapter that the ability to detect specific pathogenic microorganism at low concentration levels requires the inclusion of an enrichment protocol to amplify the pathogen's levels so that there is a high degree of confidence that at least one cell is present in the analytical unit. The level of confidence is dependent on a number of factors such as the initial level of the target microorganism in the food, the rate of growth of the microorganism in the enrichment medium, the presence of competitive microorganisms, the size of the analytical unit tested, the method of analysis, etc. The ideal solution would be to find a method for concentrating the target microorganism without producing a similar concentration of competing microorganism, altering the characteristics of the enrichment medium, or injuring the target microorganism. One of the most widely used techniques to achieve these goals is the use of immunomagnetic separation/concentration systems.

The basic approach is to attach capture antibodies specific for the target microorganism to paramagnetic beads or other solid matrices (Fig. 10.5). The antibody coated beads are then mixed with either the food sample or the enrichment broth. After thorough mixing, the sample container is exposed to a

Table 10.9 Probability of selecting a *L. monocytogenes* colonies after enrichment in the presence *Listeria innocua* and Plating on PALCAM agar (see Fig. 10.4 and text)[a]

Initial concentration in enrichment broth [Log(CFU/ml)]		Ratio of *L. innocua* to *L. monocytogenes* [Log(CFU/ml) – Log(CFU/ml)]	Probability of picking a *L. monocytogenes* colony (%)[b]			
Listeria innocua	*Listeria monocytogenes*		n = 1	n = 5	n = 10	n = 20
−3.00	−2.00	0.77	17.0	60.8	84.9	97.9
−2.00	−2.00	1.77	1.7	8.3	16.0	29.8
−1.00	−2.00	2.77	0.2	0.9	1.7	3.5
0.00	−2.00	3.77	<0.1	0.1	0.2	0.4

[a]*L. monocytogenes* and *L. innocua* appear similar on PALCAM Agar plates. The species are differentiated by selecting one or more colonies and running confirmatory tests
[b]Calculated using hypergeometrical distribution (see text)

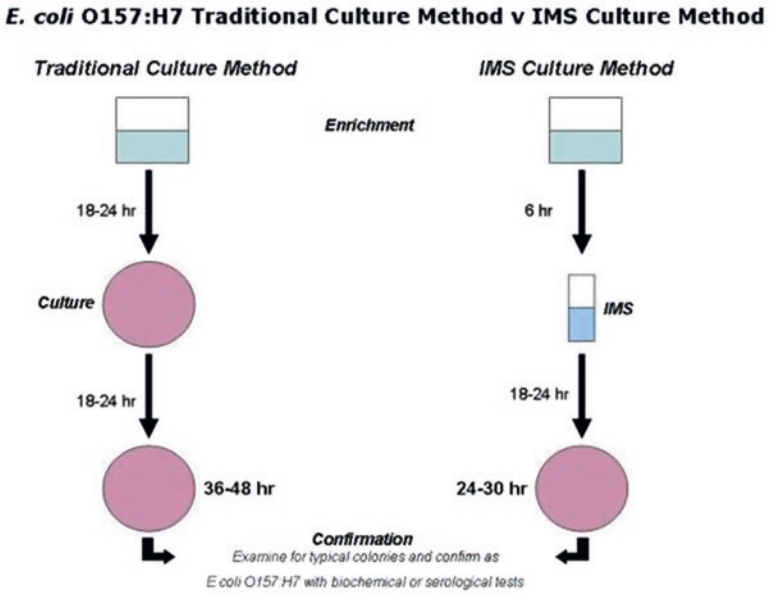

Fig. 10.5 Comparison of Traditional Cultural and IMS Culture Methods (Rapid Microbiology Newsletter, http://www.rapidmicrobiology.com/news/995h101.php)

strong magnetic field that attracts the magnetic beads and retains them at the site of the magnetic field. The original sample volume is decanted, the beads washed with buffer to remove residual enrichment broth or food homogenate. The beads with attached target cells are then re-suspended in a reduced volume, the magnetic field removed, and the concentrated suspension of cells transferred to a detection system (e.g., plating medium, PCR).

As a means of demonstrating the impact of immunomagnetic separation/concentration, we will consider a hypothetical example involving the presence of low levels of *Escherichia coli* O157:H7 in raw skim milk. If we assume that the original concentration in the milk is 10 CFU/ml and that a 10 ml sample was subjected to an immunomagnetic concentration which was 80% efficient and then re-suspended in 0.5 ml of buffer, the expected concentration would be 16 CFU/ml. If five 50-µl samples were then plated on a differential agar, the likelihood of detecting at least one positive colony and rejecting the lot is 98.2% compared to 24% probability if the original sample was directly plated without the concentration step.

During recent years there has been increased interest in developing immunomagnetic separation techniques (e.g., Pathatrix system) that examine even larger samples through the use of circulating systems in an abbreviated enrichment protocol that presents diluted food samples to antibody coated beads that are pre-adhered to magnetic field at a location in the flow of the enrichment broth. After enrichment, the beads are released from the magnetic field and detected by any of number of potential detection systems. Concentration factors of 1000- to 10,000-fold are potentially achievable in such systems. However, the effectiveness of these systems is dependent on a number of factors. Two particularly important factors are (a) the specificity and sensitivity of the capture antibody, and (b) the degree of non-specific binding (non-target microflora and/or food matrix) to the beads. Both will affect the concentration step and limit the benefits to the detection of lots that are contaminated at low defect rates. Only a limited of number of direct evaluation of immunomagnetic concentration technologies have been conducted to examine the appropriate sampling statistics that should be used with these types of technologies.

10.6 Determination of Sample Size in Non-cultural Microbiological Methods and Its Impact on the Effectiveness of Sampling Plans

The past decade has seen a substantial shift to various rapid methods based on the detection of specific genes or proteins associated with the microorganisms of concern. However, there have been only a limited number of studies that have studied the underlying sampling and methodological statistics associated with the examination of foods and water used with immunologic and genomic based technologies. However, some concepts can be inferred based on the biological principles. As discussed throughout this chapter, the sampling statistics for cultural methods is strongly driven by the fact that microorganism are distinct particles and cannot be infinitely diluted. Thus, at some point the probability that an analytical unit actually contains a microorganism will impact the probability of detection. This is overcome by taking more or bigger samples or enriching the sample. The use of genomic based methods, particularly those that rely on the detection of a single portion of chromosomal DNA, would be expected to follow a similar pattern. Thus, if an analytical unit is taken prior to DNA extraction, the limiting factor will be if a microorganism is present. Conversely, if the DNA is extracted and then the analytical unit taken, since there is effectively one DNA copy per original cell, there will be an issue of whether the analytical unit contains a copy of the target DNA sequence. The actual effectiveness of the method will be further decreased based on the effectiveness of the DNA extraction procedures and the presence of any PCR inhibitors that are co-extracted with the DNA. The efficiency of the PCR reaction and reaction volumes used in PCR reactions will also influence detection performance. This implies that effects of sample size and the need for enrichment are going to be similar to those described above for cultural methods. This supposition is supported by the limited number of studies that have compared the sampling statistics of cultural and genomic based methods (Converse et al. 2012; Copin et al. 2012). Based on these parameters, at least at the current time, achieving the sensitivities associated with food safety applications the use of such technologies will be dependent on effective enrichment techniques when the concentration of target microorganisms is below approximately 10^3 CFU/ml, depending on the size and number of the analytical units being examined. Similarly, immunologic methods have their limitations and appear to be even more dependent on understanding within method sampling limitations and the application of effective appropriate enrichment techniques. Despite recent developments in nucleic acid-based analytical methods to differentiate viable and non-viable cells, most methods do not differentiate these forms of cells but rather only detect the nucleic acid/target gene. Without an enrichment step, promoting the growth of cells,

caution has to be exercised with positive PCR-based signals and subsequent rejection/acceptance of lots based on such outcomes.

One approach to overcome this limitation is to find a genomic target which has a substantially higher number of copies. Typically, methods based on the release of 16S ribosomal DNA which is typically present at a high copy number can be applied. Again, there has been limited study of the underlying sampling statistics of these methods. With respect to viruses, particularly the foodborne norovirus and hepatitis A virus, no effective culture methods exist for their detection, therefore real time PCR is the commonly applied analytical technique. These methods do not differentiate infective from non-infective viral particles. While cultural enrichment is not possible, attempts to improve sensitivity include concentration of sample (e.g. water) or concentration of nucleic acid extracted from a food matrix (e.g. in eluent from washed fruits/berries). Another issue in assessing the lower limit of detection for methods that involve detection of RNA viruses or method based on assessing the levels of mRNA is the stability of RNA and its impact of the need to retain reference samples for subsequent confirmation.

10.7 Effect of Specificity and Sensitivity on Sampling Plans and the Operation Characteristic (OC) Curve

In many situations in the determination of the performance of sampling plans, and also for example in Chaps. 6 and 7 of this book, it is assumed that the microbiological methods work perfectly. However sensitivity and specificity do impact the operating characteristic curve (OC-curve). Especially the specificity of the testing method has a very large effect on the performance of a sampling plan, even more so for sampling plans with higher numbers of samples. Even a low rate of false positives in a method (specificity), in sampling plans with high numbers of samples, can result in a very high rejection chance of a contamination-free product batch. Therefore test methods used in sampling plans with larger number of samples should have a very high specificity (>>99%). Sensitivity has a much smaller effect on the performance of sampling plans, unless the sensitivity is very low (<0.7) (Zwietering and den Besten 2016). The effect of various values of sensitivity and specificity on the probabilities of acceptance of a batch with an actual defective rate of 0.01 are represented in Table 10.10. Furthermore the effect of sensitivity (0.99) and specificity (0.7) on a sampling plan with 60 samples is shown in Fig. 10.6.

10.8 Summary

While consideration of sampling statistics is recognized as an important factor in the effectiveness of microbiological testing of foods, the primary focus has been on the taking of food samples. However, the above examples demonstrate that sampling concepts also help to explain other methodological performance characteristics. There is a propensity for method developers to treat microorganisms as if they were chemicals. However, the particulate nature of microorganisms requires consideration of the probability that a microorganism or its components are actually in the analytical unit(s) being examined. These factors need to be considered when evaluating the effectiveness of microbiology methods, particularly when enrichment techniques are used to amplify the levels of the target microorganism. As demonstrated above, this can greatly influence the effective LOD of a method and thus the effectiveness of an overall sampling plan.

Table 10.10 Effect of the specificity (with sensitivity 100%) and the sensitivity (with specificity 100%) of a method on the probability of accepting a batch that has an actual contamination rate of 0.01 (probability of a defective in a sample P_{def})

Specificity	$n = 1$	$n = 5$	$n = 10$	$n = 20$	$n = 30$	$n = 60$
100	0.990	0.951	0.904	0.818	0.740	0.547
99.9	0.989	0.946	0.895	0.802	0.718	0.515
99.5	0.985	0.927	0.860	0.740	0.636	0.405
99	0.980	0.904	0.818	0.669	0.547	0.299
98	0.970	0.860	0.739	0.546	0.403	0.163
97	0.960	0.817	0.667	0.445	0.297	0.088
96	0.950	0.775	0.601	0.362	0.217	0.047
95	0.941	0.736	0.541	0.293	0.159	0.025
94	0.931	0.698	0.487	0.237	0.116	0.013
90	0.891	0.562	0.315	0.099	0.031	0.001
Sensitivity	$n = 1$	$n = 5$	$n = 10$	$n = 20$	$n = 30$	$n = 60$
100	0.990	0.951	0.904	0.818	0.740	0.547
99.9	0.990	0.951	0.904	0.818	0.740	0.547
99.5	0.990	0.951	0.905	0.819	0.741	0.549
99	0.990	0.951	0.905	0.820	0.742	0.550
98	0.990	0.952	0.906	0.821	0.744	0.554
97	0.990	0.952	0.907	0.823	0.746	0.557
96	0.990	0.953	0.908	0.825	0.749	0.561
95	0.991	0.953	0.909	0.826	0.751	0.564
94	0.991	0.954	0.910	0.828	0.753	0.567
90	0.991	0.956	0.914	0.835	0.762	0.581

Values for $P_{def} = 0$ and $P_{def} = 0.05$ and the calculation of these numbers is explained in Zwietering and Den Besten (2016)

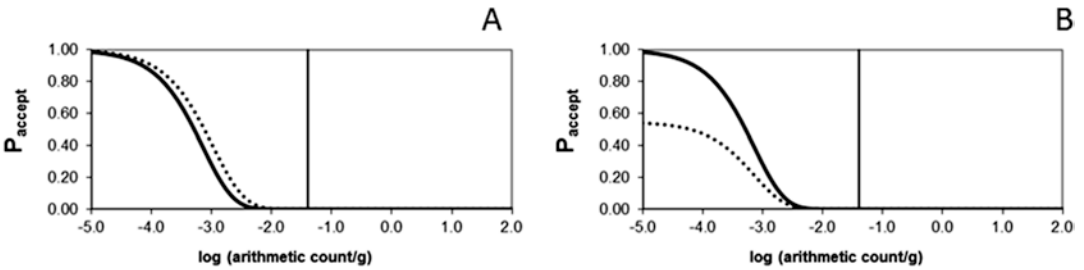

Fig. 10.6 Effect of sensitivity and specificity on the OC curve for a sampling plan for *Salmonella* with $n = 60$ (samples of 25 g) as function of log arithmetic count/g with sigma 0.8 log cfu/g and microbiological limit −1.4 log cfu/g (absence in 25 g). Panel A sensitivity is 0.7 and specificity is 1.00; Panel B sensitivity is 1.00 and specificity is 0.99. Black full line is sampling testing with ideal test method (sensitivity is 1.00 and specificity is 1.00), dashed line is sampling testing with imperfect test method

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Chapter 11
Tightened, Reduced and Investigational Sampling

11.1 Introduction

Tightened sampling involves the use of more frequent sampling and/or more stringent sampling plans than normal and is implemented when evidence suggests an increased likelihood of a hazard. Reduced sampling involves less frequent sampling, fewer samples, or smaller sample sizes than normal, and is implemented when the risk of a particular process or product is considered to be low. Investigational sampling consists of sampling to determine the direct and underlying causes of a problem.

Factors warranting tightened or reduced sampling may relate to the food product, the manufacturer, or the country of origin (Table 11.1). In tightened sampling, the sampling plans discussed in earlier chapters are no longer applied. Tightened sampling usually requires increased sample numbers (n) with other adjustments to make the sampling plan more stringent. In a 3-class plan with fixed m and M, stringency is increased by making c smaller or n larger. When 2-class plans are involved and c is 0, the sample number n must be increased to obtain a more stringent plan, assuming m is fixed. Alternatively, an increased analytical unit size may be introduced. In the case of reduced sampling, the frequency of sampling a particular food is reduced, however, the 2 and 3 class sampling plans originally specified are applied.

If the overall history is good, as evidenced by consecutive lot acceptances, then it may be appropriate and desirable to reduce the amount of inspection. This can be done by permitting smaller number of sample unit (i.e., n) than those used in normal inspection or by sampling less frequently. In some cases, this is referred to as skip-lot sampling.

Investigational (or investigative) sampling is a term used to describe sampling that is related to a known or suspected problem. Investigational sampling differs from tightened sampling in that the direct and underlying causes of a problem are sought, so that steps can be taken for corrective action and preventing recurrence. Such problems can arise from failure of a lot (or a series of lots) to pass routine inspection or from new information, such as field reports of illness or unexpected spoilage related to the product. Investigational sampling may be done to (i) confirm that a problem exists, (ii) assist in describing the nature (e.g., is it a safety or spoilage/quality problem) and extent of a problem, (iii) provide information on possible sources of a problem, (iv) help decide what to do with a product (e.g., is the entire lot unacceptable or is the contamination limited to a certain portion), and (v) prevent the problem from recurring. When this information is available, decisions can be reached on segrega-

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International Commission on Microbiological Specifications for Foods (ICMSF),
Microorganisms in Foods 7, https://doi.org/10.1007/978-3-319-68460-4_11

Table 11.1 Circumstances warranting tightened or reduced sampling of food

Warranting increased frequency of sampling and/or a more stringent sampling plan	Warranting reduced frequency of sampling
The food operation	
An audit indicates the operation does not have an adequate system of controls based on GHP and HACCP	The operation has an effective system of control based on GHP and HACCP
Records indicate a deviation at a CCP in the HACCP plan has occurred	Records indicate the operation is under control
Information indicates the operation has used an ingredient from a source that has caused problems in other similar operations	
Food from the operation has recently been involved in illness Line commissioning (e.g., new line installed) in aseptic manufacturing	Food from the operation has a favorable history of safety
The food and raw materials	
Testing of finished product or product contact surfaces indicates pathogen contamination e.g. 'positive' sample	
The composition of the food differs from other foods of the same type and an increase in a hazard is likely to occur under expected conditions of storage and distribution	The composition of the food differs from other foods of the same type and the potential hazards will decrease or be eliminated during expected conditions of storage and distribution
Previous tests are frequently unsatisfactory	Previous tests are satisfactory
Routine tests for indicators have revealed a trend toward increased contamination	Routine tests for indicators show continuing control
The food has a history of being a cause of foodborne illness.	Rarely involved in foodborne illness
The food has been found to be a source of a newly emerging pathogen or new type of an existing pathogen	
Circumstances suggest involvement of this type of food or common raw materials in a current/recent outbreak or contamination event	
A food that traditionally has been for the general public is to be directed toward a sensitive population, where it could present a health risk	Not primarily intended for sensitive populations
New type of food or new formulation with reason to be concerned about a microbiological hazard	The parameters necessary for controlling foodborne illness are well known and widely applied
Examination results from different laboratories are in conflict and disposition of the food is in question	
Country or region of origin	
Food control systems are in question	The food control systems are known to be equivalent for control of the food or ingredients in question
Endemic or epidemic situations exist that increase concern for consumers of the food Evidence (e.g., from alert systems) of contamination with microbiological hazards from particular regions	Endemic or epidemic situations do not exist that would increase concern for consumers of the food

tion, blocking and recalling product (if that has not taken place already), salvaging of unaffected batches and the status of further production.

It is important to note the difference between investigational sampling and the type of sampling described elsewhere in this book. Attribute or variables sampling allows for detecting effects; whereas investigational sampling seeks to determine causes. Random sampling is not an effective or efficient way to carry out investigational sampling. The success of investigational sampling depends greatly on the expertise of the investigator, the investigator's knowledge of microbiology, the product, and process involved in its manufacture, equipment design and the conditions of storage, distribution and use.

The attributes and variables plans previously discussed for lot acceptance are intended for routine application to lots or consignments presented for inspection at various stages prior to sale to the public. Two and three-class attributes plans are used for regulatory, port-of-entry, and other receiver-oriented situations, where little information is available concerning the microbiological history of the lot. Variables plans can be used when the distribution of microorganisms (e.g., APC, pathogens, indicator microorganisms) is known. They may be applicable to in-plant quality control, where these assumptions can be properly verified. In either case, the emphasis in lot acceptance sampling plans is to do the minimum amount of work necessary to obtain the degree of security that each plan can provide.

When a potential problem has been identified (e.g., re-contamination of a product with *Salmonella or Listeria monocytogenes*), further examination into the nature and extent of the problem is frequently required (i.e., investigational sampling). Such might be the case if a lot rejected under routine inspection is in dispute or if the cause of a problem is being sought. For example, routine acceptance sampling may have indicated occasional recontamination in a plant from an increased rejection rate of lots over the history. A larger random sample may then be taken from the rejected lots to confirm that a problem exists and estimate the extent and distribution of the contamination. Emphasis may then shift to the processing plant where samples could be taken from various sites in an attempt to locate the source of contamination (e.g., certain processing equipment, point(s) of moisture ingress/condensation in a dry environment, a particular ingredient). It is necessary to identify the contamination source so that appropriate measures can be taken to correct the problem. It is important to take an evidence-based approach during investigations and not to jump to conclusions. In another situation, analysis of rejected lots may show that contamination is associated with certain production or filling times (e.g., first few hours, after breaks, following equipment repair). This information can be used to focus on the circumstances that may be causing contamination and how it may be prevented. For situations with very low-prevalence contamination, it can prove difficult to find more than one positive sample. A positive result must never be ignored unless there is a clear reason to suspect contamination during sampling, transport to laboratory or laboratory error.

The described hypothetical investigations have used sampling at various stages in a food operation. Such sampling is rarely random. Biased sampling is much more efficient because it takes advantage of prior knowledge of the operation, visual observations, and logic that may lead the investigator to sample those locations that are most likely sources of the problem, e.g., a conveyor or the first containers filled in a 'clean filling' operation following plant cleaning or a line stoppage. Random sampling of easily accessible (and thus easily cleaned) sections of the equipment may ignore product accumulation in less easily accessible areas, such as hollow gears that drive conveyors or valves controlling product flow in-line, which escape proper cleaning and disinfection. A common approach to investigational sampling used by industry is to collect in-line samples of product at selected stages of processing with a bias toward those most likely to be contaminated or indicative of the source of a problem.

Biased sampling is also useful for suspected lots of food that are in storage or have been received at a location. For example, it may be evident from pack or container appearance that a dried product may have become wet due to a failure in pack integrity and ingress of moisture, or that there is container leakage during transport evidenced by swollen or blown packs. In this case, it may be appropriate to take samples from the area most likely to have become wet (e.g., the top packs on a pallet) or from areas where packs may have been physically damaged (e.g., on edges of pallets). Indications that a non-random problem may have occurred include transit pack damage, pallet collapse, or evidence of spoilage or discoloration in the outer packs suggesting abusive holding conditions. Other approaches to sampling, such as stratified, systematic, cluster, or various combinations of these, may also be used. Random and systematic sampling approaches can be compared to detect localized contamination that might occur within a batch of solid, semi-solid or powdered food. Microorganisms present in the

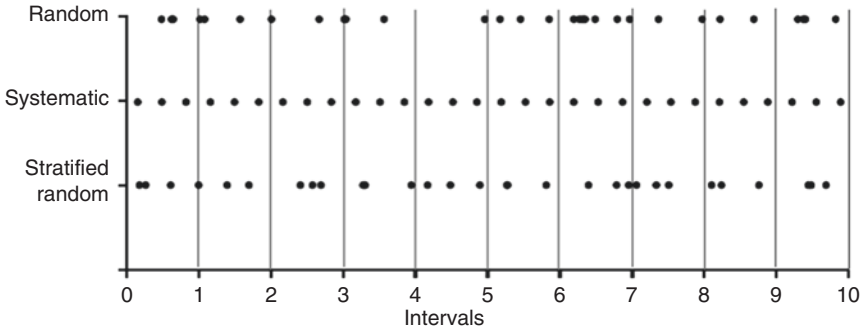

Fig. 11.1 An illustration of random, systematic and stratified random sampling of 30 sample units

localized contaminated fraction can be assumed to be randomly distributed and in such cases, systematic sampling is preferred over random sampling for detecting such contamination (Habraken et al. 1986; Rivas Casado et al. 2009). In the case of systematic contamination, such as may arise from a contaminated filler head, it might be possible to consistently miss the contamination when using systematic sampling. In this case, stratified random sampling, another type of systematic sampling, would be more appropriate as shown in Fig. 11.1 (Jongenburger 2012). This figure illustrates three sampling strategies to draw 30 sample units using random, systematic and stratified random sampling. In stratified random sampling, three random sample units are drawn from each interval or stratum. In a moving stream of product, these intervals could be a specific weight or a time interval that the product passes a specific point. Since stratified random sampling combines the qualities of systematic and random sampling, this strategy is preferred and is the most appropriate to use in cases of both clustered and systematic contamination.

Generally, once the purpose of sampling has been established, the most efficient type of sampling applicable to this purpose is chosen. Such topics are beyond the scope of this book and are statistically complex, but an excellent discussions are found in Cochran (1977) and Jarvis (2007).

For inspection by attributes, specific rules have been established (ISO 1999) to enable switching from one category of sampling procedures to another e.g., from normal to tightened and vice versa, and normal to reduced and vice versa. The rules are intended to provide a basis for protecting the consumer (e.g., by tightened sampling) if a deterioration in quality is suspected or detected, and an incentive to reduce inspection costs (by switching to reduced sampling) when good quality and safety are consistently achieved. However, this standard was not developed specifically for microbiological testing and there are more relevant examples available of reduced or tightened sampling based on the hazard, and whether this increases, decreases or does not change, and the intended use. Table 11.2 illustrates the ICMSF recommended sampling plans for *Salmonella* in dried milk, where the plan chosen is dependent on whether the expected handling, storage, and use of the product will change the level of hazard in the product. This also considers susceptibility of the target consumer group. A detailed risk assessment for *Salmonella*, FAO/WHO (2002) compared the attack rates of *Salmonella* for children less than 5 years of age, against those for the rest of the population in the outbreak database. Although some indication for a difference in attack rates for the two populations had been noted in two of the outbreaks examined, this analysis did not reveal an overall trend of increased risk for this subpopulation. The severity of illness as a function of patient age was not evaluated, and it was concluded that the database of information was insufficient to derive a quantitative estimate for this factor. Nevertheless, there are some reports of increased severity in infants e.g., Olsen et al. (2001) reported a 4–13-fold higher rate of invasive disease in young children compared to other age groups. At 139.4 cases per 100,000 infants, the incidence of salmonellosis (from all sources) among infants

was reported to be more than eight times greater than the incidence across all ages in the United States of America in 2002 (CDC 2004). Salmonellosis incidence patterns are similar in the United Kingdom. Infants experience the highest rate of infection, with 181 cases per 100,000 infants (Skirrow 1987). It is unclear whether the increased rate among infants results from greater susceptibility, or whether infants are simply more likely than persons in other age groups to seek medical care or have stool cultures performed for symptoms of salmonellosis (FAO/WHO 2006).

For milk powder, there may be no change in the level of *Salmonella* when the powder is tested at the factory, at the port of entry, or when the powder is reconstituted for serving, and therefore case 11 is appropriate. However, if the powder is intended for a high risk population, such as infants, then the number of samples to be tested increases, with case 15 being most appropriate. Using the approaches of Foster (1971) and Legan et al. (2000) and assuming a log normal distribution and standard deviation of 0.8, performance of the sampling plans can be related to the geometric mean concentration of bacteria that could be detected with a 95% probability. These criteria are applicable where no information is available on the history of the lot or the supplier's control system. Table 11.2 shows two class sampling plans for *Salmonella* in dried milk, considering level of hazard and susceptibility of target consumer group.

11.2 Application of Tightened Sampling and Investigational Sampling

Tightened and investigational sampling generally are applied to situations where there is an increased level of concern or perceived risk, such as when a process deviation has occurred, a performance criteria have not been met, a product has failed to meet microbiological criteria, the food is from an operation with a history of inconsistent control, the food is from a region where there has been a recent increase in illness involving the same or similar type of food, or an environmental monitoring indicator has shown that the equipment used to produce or package the product did not meet acceptable hygienic criteria. Tighter sampling also may be warranted when there is insufficient knowledge about a particularly sensitive ingredient or a food is intended for a sensitive population and, thus, there is increased concern for making a correct decision as to its acceptability. For example, shipment from a new supplier or from a country in which the hygienic and manufacturing practices are not known may warrant increased sampling. These circumstances may confront both industry and regulatory agencies, and both may employ tightened sampling and investigational sampling. However, the objectives and approaches may differ. Control authorities are primarily interested in protecting the consumer. Industry will have that same goal but also must protect the economic interests of the company. Both seek to differentiate acceptable from unacceptable product, but it is also critical for industry to know why a problem may have occurred and how it can be corrected.

Control authorities may have less information available than industry about the source and production of a particular suspect lot, and in many cases would place the onus of identifying the affected lots (and associated sampling to establish this) on the industry. Control authorities will often undertake investigation of the processing/manufacturing environment and in some cases, e.g., to inform risk management decisions, undertake or request further testing to be carried out. Industry, with more information at its disposal, may choose to use an investigational approach deliberately employing biased sampling. Targeted sampling of finished product is often more efficient than random sampling when contamination might be associated with a time or sequence of production; such as the first product produced after a shutdown, shift change, ingredient switch, mechanical repairs, etc.

Occasionally, control authorities may also employ tightened sampling or investigational sampling. For example, if an outbreak involving a certain product (or products) produced by several producers, the control authority will have a much broader focus than the individual manufacturer, and may have

Table 11.2 Two class sampling plans for *Salmonella* in dried milk, considering level of hazard and susceptibility of target consumer group, assuming a standard deviation of 0.8, log normal distribution, and where $c = 0$. Lots having the calculated mean concentration or greater will be rejected with at least 95% probability

Test	Type of hazard	Potential change in hazard	Case	Number of sample units	Mean concentration
Salmonella (normal, routine)	Serious	Conditions reduce hazard	10	5	32/1000 g
		Conditions cause no change in hazard	11	10	12/1000 g
		Conditions may increase hazard	12	20	5.4/1000 g
Salmonella for high-risk populations, e.g., infants	Severe	Conditions reduce hazard	13	15	7.4/1000 g
		Conditions cause no change in hazard	14	30	3.6/1000 g
		Conditions may increase hazard	15	60	1.9/1000 g

more epidemiological information at its disposal to help identify the source of the problem. In this case, the control authority may apply both biased sampling as well as tightened sampling, using random samples to determine which manufacturer or ingredient supplier is the source of the outbreak. Industry would be much more likely to employ tightened sampling to differentiate between acceptable and unacceptable lots of ingredients and/or finished product.

Another example of tightened sampling can be found with aseptically packed products that are sterilized in-line and then filled into sterile containers. It is common practice for products to be sampled at high frequency (e.g., 30,000 packs per 300,000) during commissioning of new lines and also following problems associated with contamination events (also see Chap. 13). Once evidence is available that the (contamination) defect rate is below the acceptable level (commonly used in industry, equal to or less than 1 in 10,000), then the sampling frequency can be reduced (see below).

Investigational sampling is also used to determine whether a contamination problem is a safety or spoilage issue and how much product is at risk, by identifying where and when the problem first arose. This is a particular concern when dealing with highly perishable foods with a short shelf life (see Chap. 17). Speed of testing is often of paramount importance in such situations, since product may have been released to the market and therefore rapid test procedures play an important role here. Other considerations that are important in the choice of test method employed include sensitivity, specificity, reproducibility and repeatability (see Chap. 10). Test methods should be properly validated and fit for purpose. In the case of infectious agents, the ability to detect low levels of pathogens is usually important and such tests commonly involve some form of non-selective pre-enrichment, followed by transfer to a selective enrichment and then detection/identification by purification and testing of phenotypic characteristics or confirmation through the use of specific immunological methods or molecular techniques, such as polymerase chain reaction (PCR) based tests.. The type of test used has an impact on the number of samples that can be tested. When testing is 100% effective, the probability of detection simply depends on the total quantity of sample tested and not on the number of individual tests (Jarvis 2007). The more samples units taken (and thus total quantity analyzed) improves the likelihood that the organism is present in the sample. However, it is widely acknowledged that test procedures may not be able to detect target organisms present at a low level, particularly if they are injured, and therefore it is rarely the case that recovery of microorganisms is independent of the volume being tested. Pooling can result in decreased sensitivity due to for example presence of background microbiota. Testing of larger quantity samples, as might be the case with tightened sampling (see below), will be less sensitive than testing a greater number of individually

smaller samples. Hence, there may be a preference to increase the number of sample units. While information on the likelihood of detecting positives with modified sampling plans can be provided through application of tools such as the ICMSF spreadsheet (Legan et al. 2000), such tools do not normally take account of the real detection limits of particular test procedures and therefore these need to be considered separately. To address this further, see the information presented in Chap. 10.

Investigational sampling may include environmental testing, to determine the source of contamination and if similar types of organism are recovered, molecular typing of the strains would be required to establish if they are related to the strain(s) isolated from product. More details of the sampling plans used in investigational sampling are provided in Chap. 12.

Tightened and investigational sampling can also be important in the design and applications of GHP and HACCP. Tightened sampling, involving many more samples than would be practical for routine testing, can be used for process validation or as part of the HACCP plan verification. Investigational sampling may be required to identify areas in a plant where GHP needs to be changed to eliminate an organism of concern (e.g., more efficient disinfection techniques).

Failure to meet the critical limit (i.e., process criterion) for a CCP may necessitate tighter sampling of a lot. Experience with the food, the likely occurrence of a hazard, and severity of the hazard(s) will influence the decision to sample the lot or to apply another option (e.g., reprocessing, destroying the lot) .

Suspicion that a food may contain a microbiological hazard may arise from a variety of sources of information:

- a hazard may have been detected in other lots from the same operation,
- an audit of an operation or an alert from a CCP has revealed questionable control,
- a consumer complaint may have raised suspicion about the food,
- complaints may be received from the trade or from the sales force,
- social media reports/trends,
- an ingredient has recently been implicated as a source of illness,
- an unfavorable trend in a hygiene indicator has been detected.

These and other circumstances (Table 11.1) may lead to tightened sampling until accumulated evidence indicates this is no longer necessary.

Additionally, application of both tightened and investigational sampling are commonly seen in legal cases, where evidence of contamination is sought (see Chap. 9).

11.3 Tightened Sampling Plans

One possible means to make a sampling plan more stringent is to increase the sample number (n) that is collected and analyzed. When 2-class plans are involved (i.e., $c = 0$) and m is fixed, the number of analytical units (n) must be increased to obtain a more stringent plan. Another option for 2-class sampling plans is not to change n but to increase the size (m) of the analytical unit (e.g., from 25 g to 100 g) for testing. The spatial distribution and independence of the defectives throughout the lot will influence whether this option will increase confidence of detecting a defective sample unit within the lot and, thereby, lead to rejection of the food. When 3-class plans with fixed m and M are involved, acceptance criteria can be made more stringent by making c smaller or n larger.

Typically, a tightened sampling plan will involve an increase in number of samples taken (i.e., increasing n). The 2-class plans shown in Table 11.3 give an indication of how the performance is impacted by changing n. This is further illustrated in Table 11.4, in which the probability of detecting one or more positive samples in batches with very low occurrence of defectives is given. Thus, if one

Table 11.3 Probability of Acceptance (P_a) for Two-Class Sampling Plans with $n = 10$ to $n = 5000$ with $c = 0$

Composition of lot		P_a for 2-class sampling plans with $n = 10$ to $n = 5000$ with $c = 0$											
% acceptable	% defective	10	20	30	50	100	200	300	500	1000	2000	3000	5000
99.9	0.1	0.99	0.98	0.97	0.95	0.90	0.82	0.74	0.61	0.37	0.14	0.050	0.007
99.8	0.2	0.98	0.96	0.94	0.90	0.82	0.67	0.55	0.37	0.14	0.018	<0.001	<0.001
99.7	0.3	0.97	0.94	0.91	0.86	0.74	0.55	0.41	0.22	0.050	<0.001		
99.6	0.4	0.96	0.92	0.89	0.82	0.67	0.45	0.30	0.13	0.018			
99.5	0.5	0.95	0.90	0.86	0.78	0.61	0.37	0.22	0.082	0.007			
99.4	0.6	0.94	0.89	0.83	0.74	0.55	0.30	0.16	0.049	<0.001			
99.3	0.7	0.93	0.87	0.81	0.70	0.50	0.25	0.12	0.030				
99.2	0.8	0.92	0.85	0.79	0.67	0.45	0.20	0.090	0.018				
99.1	0.9	0.91	0.83	0.76	0.64	0.40	0.16	0.066	0.011				
99	1	0.90	0.82	0.74	0.61	0.37	0.13	0.049	0.007				
98	2	0.82	0.67	0.55	0.36	0.13	0.018	0.002	<0.001				
97	3	0.74	0.54	0.40	0.22	0.048	0.002	<0.001					
96	4	0.66	0.44	0.29	0.13	0.017	<0.001						
95	5	0.60	0.36	0.21	0.077	0.006							
94	6	0.54	0.29	0.16	0.045	0.002							
93	7	0.48	0.23	0.11	0.027	0.001							
92	8	0.43	0.19	0.082	0.015	<0.001							
91	9	0.39	0.15	0.059	0.009								
90	10	0.35	0.12	0.042	0.005								

Table 11.4 The probability of obtaining one or more defectives in a sample of n sample units with proportion p of the lot defective

Number of sample units examined per sample, n	Probability of obtaining one or more defectives with the following proportions (p) of defective			
	0.01	0.001	0.0001	0.00001
200	0.87	0.18	0.02	0
1000	1.00	0.63	0.10	0.01
2000	1.00	0.86	0.18	0.02
3000	1.00	0.95	0.26	0.03
4000	1.00	0.98	0.33	0.04
5000	1.00	0.99	0.39	0.05

unit in every 1000 were contaminated, even a sample of $n = 500$ would result in a 61% chance of accepting the lot; that is, there is only a 39% chance of finding one or more contaminated units with $n = 500$ when the contamination rate is 0.1%. Clearly, such a probability of detection/rejection would be unacceptable for highly toxic contaminants or severe microbiological hazards. Calculation of lot acceptance and likelihood of detecting positives using a tightened sampling plan is made easier through use of the ICMSF spreadsheet (Legan et al. 2000) but other factors related to the test procedure used still need to be considered (see Chap. 10).

It is unlikely that sampling plans with n between 300 and 5000 will be used for microbiological testing due to laboratory effort and cost. It may be possible, however, to conduct simple non-microbiological tests on large numbers of sample units or simpler tests using a pH indicator in broth (microbiological medium), such as is used in commissioning trials for aseptic lines. The options for testing a batch of canned food may be to conduct a simple test such as pH measurement, can seam analysis, or examination for vacuum or swelling or microscopic examination to identify potentially hazardous cans. Examination for vacuum in cans can be carried out using tap-tone systems that measure the vacuum by lid deflection using a magnetic field and while these are usually a component of on-line control systems, they may also be used to inspect cans that have been held for a period of time. While microbiological analysis involving conventional culture media may be very effective in identifying contamination, a large number of examinations are not practical. A measurement of product pH may be less likely to identify a container in which growth has taken place, but large numbers of analytical units can be examined. As contamination may occur at a relatively low rate, a simple test that allows examination of a large number of units would be more effective.

A common question is how many analytical units would need to be tested to provide some level of confidence that a lot will be rejected if it is hazardous to consumers. In general, the number of samples necessary will depend on the level of defective units that can be tolerated, and the desired confidence to detect a defective lot. If, for example, a 2-class attributes sampling plan is applied as a "zero tolerance" plan (i.e. $c = 0$), a defective lot would be detected as soon as at least one sample unit is defective. When sampling a given lot, the probability of finding at least one defective unit depends on the actual percentage of defectives in that lot and on the number of sample units drawn. Hence, to select a sampling plan and derive the number of sample units (i.e., n) two decisions are first necessary: a decision on the percentage of defective units in a lot that defines a "defective lot", and a decision on the required confidence level (e.g. 95%), i.e., on the desired probability of detecting at least one defective unit, if the lot is defective (see Chap. 6).

For such 2-class plans, Table 11.5 provides guidance on the number of random sample units that would be needed to provide 90, 95 and 99% confidence that at least one defective sample unit will be detected in lots with defective levels ranging from 0.1% to 50% (Cannon and Roe 1982). For example, if it is desired to have 95% confidence that a lot does not have 2% or greater defectives, then it

Table 11.5 Number of sample units required for 90%, 95% and 99% confidence of detecting at least one defective sample unit in lots with defective levels ranging from 0.1% to 50%

Desired confidence level	Definition of defective lot (%)	Number of sample units
90%	50	4
	20	11
	10	22
	5	45
	2	114
	1	230
	0.1	2302
95%	50	5
	20	14
	10	29
	5	59
	2	149
	1	299
	0.1	2995
99%	50	7
	20	21
	10	44
	5	90
	2	228
	1	459
	0.1	4603

would be necessary to collect 149 sample units from the lot. To increase the level of confidence to 99% then it would be necessary to collect 228 sample units.

If the percentage of defectives that should be detected is 10%, then 29 sample units would be required for the same level of confidence. As the proportion of defectives falls below *ca* 1%, the intensity of sampling and testing increases to levels that are impractical in a microbiology laboratory. In this case, alternative testing should be used (see above).

In another form of tightened sampling, certain foods may be sampled at greater than the established 'normal' frequency. The sampling plans discussed in Chaps. 6, 7, and 8 on a lot basis, which for "hold and test" programs generally assume that every lot is sampled. The frequency of sampling is another factor that needs to be considered when establishing a microbiological sampling program. There is no strict definition of a lot, though with many commodities it is considered to be the food produced from one "clean-up" to next. In practice, however, microbiological criteria and sampling plans are commonly established for a wide variety of foods, but in many instances only a limited number of lots are actually sampled. Conversely, in other instances the lot are defined as food produced during a specific period, e.g., every 2 h of production. The established microbiological criteria define what is considered acceptable and what the food product should comply with. They are often applied on an as-needed basis (see Tables 4.3 and 9.1). In practice, the frequency of testing a food can range from none, or rarely, to 100% of the lots. Increasing the application of an established sampling plan beyond that which is routinely applied would be an additional form of tightened sampling.

11.4 Example of the Influence of Sampling Plan Stringency in Detecting and Identifying Defective Lots, and Re-establishing Under Control Conditions

Sampling plans of different stringency that are applied at different steps in the food chain or in an operation may reveal differing levels of control.

Example: A drink mix containing nonfat dry milk (NFDM) intended for infants is prepared by mixing dry ingredients. No kill step is applied. In this example, the ingredients have been found negative for *Salmonella* using a case 14 sampling plan (see Chap. 14). Yet, the finished product was found to be positive using the same sampling plan. The ingredients were usually of a much greater quantity than the lot size of the product such that a lot of NFDM tested at case 14 ($n = 30 \times 25$g) would be used in several smaller lots of drink mix, also tested at case 14. The NFDM made up about 60% of the finished product. Thus, a greater quantity of NFDM was being tested as a component of the finished product than was tested to approve its use as an ingredient. This is illustrated in the following hypothetical example:

- Lot size of NFDM = 100,000 kg
  - Each lot of NFDM sampled at case 14 ($n = 30$).
  - Thus, 30 × 25 g = 750 g tested per 100,000 kg.
- Lot size of drink mix = 10,000 kg
  - Each lot sampled at case 14 ($n = 30$).
  - Thus, 30 × 25 g tested = 750 g per 10,000 kg.
- Drink mix = 60% NFDM or 6000 kg NFDM per 10,000 kg lot of drink mix

100,000 kg of NFDM was used to make 16.67 lots of diet drink.

  - Since 750 g was tested per lot of drink mix, this meant that a total of 1250.5 g was analyzed per 16.67 lots.
  - Thus, 60% NFDM × 1250.5 g of drink mix = 7501.6 g of NFDM analyzed as a component of the drink mix.

In this example, 750 g of NFDM was sampled and tested for acceptance as an ingredient but a total of 7501.6 g was sampled and tested as a component of the finished drink mix. Thus, the NFDM was being tested at about a 10-fold greater stringency as a component of the finished product than when being tested for acceptance as an ingredient. Such experience should lead to a tightened sampling of the NFDM as ingredient and/or a change of suppliers.

As demonstrated in the above example, the potential exists for low level contamination to be missed by one sampling plan but detected by another more stringent sampling plan applied at different step in a food operation or elsewhere in the food chain. The choice of stringency of an attribute sampling plan applied to an ingredient must take into consideration lot size, use level, and microbiological criteria applied to the finished product. Further, if microbiological records are reviewed for the purpose of problem solving, negative results for ingredients should not eliminate them as a possible source of contamination until the balance between ingredient and finished product sampling plan stringency is considered.

For the identification of which lots may be affected by a problem, other information may already be available to indicate when the process started to fail and this will inform more stringent sampling plans that can be used to identify/confirm which particular lots are affected. Examples of such information include process control charts (see Chap. 13), visual observations of physical defects (e.g., swelling) in particular packs and use of time/date coding, CCP monitoring information, etc. Using

such information, more stringent sampling plans may be applied to particular lots to establish whether these are affected and also to confirm the failure rate. It is possible that the existing information (e.g., swollen containers) indicates a particular rate of failure in particular lots, but there may be other lots affected, perhaps with a lower and continuously decreasing rate of failure that is not immediately obvious because of the time required for the physical defect to become apparent. It is important to identify all of the lots affected so that appropriate risk management decisions can be taken and so that unnecessary costs of recovering unaffected lots are avoided. It is also possible that the apparent rate of failure is underestimated based on initial observations, due to these being associated with a particular type of contaminant that is not present in other packs that may also be affected, but with microorganisms not able to produce the same obvious signs of contamination. This is another important reason to carry out more stringent microbiological testing to identify all affected lots. This is particularly important when communicating recalls to the public; expanding recalls after an initial announcement is typically viewed negatively by the public.

To establish that the process is back in control following a contamination event, application of a more stringent sampling plan is necessary. In some contamination situations, the direct cause may never be identified and although actions may be taken to prevent recurrence, it is not certain that the problem has been rectified and in these cases, more stringent sampling can provide valuable information and assurance. Through application of a more stringent sampling regime, there is more certainty that the defect rate is below the acceptable limit.

11.5 Selecting the Sampling Plan According to Purpose

Before choosing a sampling plan, the objective of the sampling should be clearly defined. Is the sampling intended to differentiate between acceptable and unacceptable product or to investigate and discover the cause of a problem? The stringency of the plan and the use of biased and unbiased sampling will depend greatly on the objective of the sampling.

One of the most difficult situations in choosing a sampling plan for investigational or acceptance sampling is when there is "zero tolerance" for the attribute being tested, such as *Salmonella* and *E. coli* O157:H7 in a RTE food. No sampling plan, short of 100% analysis of the food, can assure complete absence of the defect. Thus, the investigator is faced with a situation where a decision must be made with regard to what stringency is adequate, but still practical to perform. Plan selection is much easier when a Food Safety Objective (FSO), Performance Objective (PO) or other established limit exists. The plan stringency needed to detect levels of the defect can then be determined. Sometimes the established limit may be too low to be practically sampled and tested for either investigational purposes or to differentiate between acceptable and suspect product. However, the investigator can at least determine the plan stringency that would be needed, even if it is not practical.

Even when the official regulatory position is "zero tolerance" there may be prescribed sampling plans that are widely used. Such is the case with *Salmonella* in the U. S. The United States Food and Drug Administration has established a "zero tolerance" for *Salmonella* in most processed foods, yet employs sampling plans equivalent to ICMSF cases 13–15, referred to as categories I, II, and III in the FDA Bacteriological Analytical Manual. Even at the highest stringency ($n = 60$), a lot containing 2% defectives would test negative 30% of the time. It is not uncommon in the U.S. to select a plan with a higher stringency to provide some confidences that the defect could be detected with greater sensitivity than if the normal attribute plan were used.

The population to be sampled must also be determined and must take into account the potential sources of the problem. Consider an example where three slicers are used to slice three varieties of meat that are then assembled to provide a package containing a portion of each. If one slicer has not

been cleaned properly, it could be a source of contamination, particularly for the first half hour or more of slicing. A sampling plan that does not select samples from each slicer, or does not include a sufficient number of samples to be relatively certain to include product from each slicer, will fail to detect the source of the problem. Clearly, if the samples can be associated with a specific slicer, a biased sample that assures the defective slicer is represented will be more efficient than a random sample of a sufficient number to be relatively certain each slicer is represented. This is especially the case if the contamination may be time related and one would like to have samples from each slicer for each time segment.

11.6 Reduced Sampling

Conditions that can lead to reduced sampling frequency may relate to the food or the source of the food (Table 11.1). For example, reduced sampling is warranted when an audit of a food operation as in Chap. 4 leads to the conclusion that a good or excellent food safety management system is in place. In addition, an ongoing sampling regime can, over time, provide a level of confidence that will allow reduced testing, indicating excellence in the manufacturing operation. Furthermore, foods of low risk (e.g., pathogens die or cannot multiply, the product has a long history of safety use) could be sampled at a lower frequency.

Reduced sampling commonly involves sampling at a lower frequency, sampling the same number of sample units over a longer period of time or, if circumstances warrant, not at all. When lots are sampled, however, the sampling plans described in Chaps. 6, 7, and 8 are recommended. That is, the acceptance criterion is comparable to that for the corresponding plan for normal inspection. It is important to note that the discriminatory ability in reduced sampling is less than that under normal inspection. The reduced level of sampling may be used until evidence suggests that normal sampling (or tightened) should be reintroduced. Relaxing the rate of sampling can include skipping sampling of some lots altogether, freeing up valuable resources that can be used in other areas. Skip-lot sampling (also known as a cumulative results plan) is a well-established procedure for reducing sampling where the reduction in effort is achieved by determining at random, with a specified probability, whether a lot presented for inspection will be accepted without inspection. Skip-lot sampling plans (reduced sampling) and their statistical basis for such plans are described below.

11.6.1 Skip-Lot Sampling

Skip-lot sampling is implemented by first designing a single sampling plan that specifies the risks (see Chap. 6). This is referred to as "the reference sampling plan." This reference plan is used to start normal lot-by-lot testing. When a pre-specified number of consecutive lots are accepted, a switch is made to sampling only a fraction of the reference sampling plan. The selection of the members (lots) of that fraction should be done at random. When a lot is rejected for whatever reason, the initial sampling plan (reference sampling plan) is resumed or a more stringent plan is implemented until confidence is regained that performance has returned to an acceptable level.

Skip-lot acceptance sampling is a procedure that allows for some lots (samples) to be *skipped* from testing. Various ANSI/ASQC (ASQC 1996) standards define *skip-lot sampling inspection* as inspection in which some lots in a series are accepted without sampling when the sampling results for a stated number of immediately preceding lots meet the stated criteria. That is, testing of each lot (based on the specified reference plan) occurs until *i,* the number of successive lots, have been found acceptable,

after which only a fraction of lots, called *f*, are selected for inspection, thereby skipping inspection on the fraction $(1 - f)$ lots. The primary methods for various skip-lot procedures are described by Dodge and Perry (1971) and Perry (1973). This mode of sampling is cost-saving in terms of time and effort. However skip-lot sampling should only be used when it has been demonstrated that the quality of the submitted product is very good or excellent.

11.6.1.1 Implementation of Skip-Lot Sampling Plans

A skip-lot sampling plan can be implemented as follows:

1. Design a single sampling plan by specifying the alpha (type I errors) and beta (type II errors) risks and the consumer/producer's risks. This plan is called "the reference sampling plan" (see Chaps. 6 and 7).
2. Start with normal sampling inspection, using the specified reference plan.
3. When a pre-specified number, *i*, of consecutive lots are accepted, switch to inspecting only a fraction *f* of the lots. The selection of the members (lots) of that fraction is done at random as done with the reference plan.
4. When a lot is rejected under the skip-lot plan, return to normal inspection as described under the reference plan.

The skip-lot procedure is governed by two equations, the probability of acceptance from the reference plan, P_{ar}, (which is equivalent to P_a described in Chap. 6) a function of *n*, and *c,* and the probability of acceptance under the skip-lot plan, P_{asl}. The P_{ar} equation is described in Chap. 6, while the P_{asl} equation is given here for convenience of discussion.

$$P_{asl}(f,i) = \frac{\left[fP_{ar} + (1-f)P_{ar}^{\,i}\right]}{\left[f + (1-f)P_{ar}^{\,i}\right]} \tag{11.1}$$

As given in Eq. 11.1, the skip-lot probability of acceptance is a function of *f*, *i*, and the acceptance probability of the reference plan (P_{ar}). In almost cases the P_{asl} will be greater than the P_{ar} and this should give a note of caution. Because this is true, one should be cautious in the application of a skip-lot plan. One just needs to calculate P_{ar} and P_{asl} and then compare them to make an assessment of alpha and beta probabilities to determine the best skip-lot plan to follow for their operations situation. Table 11.7 gives the calculations for a selected combination of skip-lot plans.

In general there is not a large difference between P_{ar} and P_{asl}. However, the largest differences are noted for reference sampling plans involving $c = 0$. This would be a logical conclusion recalling earlier discussions (see Chaps. 6 and 7) about the alpha and beta probabilities of such plans. For this reason it is suggested to either not consider skip-lot sampling for reference plans where $c = 0$ or to be extremely cautious in selection and implementation. Additionally, Table 11.6 shows that for a given *i*, as *f* gets smaller, the greater P_{asl} is, a desirable quality of a selected skip-lot sampling plan (Stephens 2001).

In addition to P_{asl}, other measures of the success of selected skip-lot plans are average sample number (ASN) and average run length (ARL) to detect an abrupt deterioration of quality (i.e., how many samples does it take to detect a change in quality, ARL), the average outgoing quality (AOQ) and the average outgoing quality level (AOQL), (NIST 2012).

The average sample number (ASN) (or average amount of inspection required) is important to determine sampling cost reductions. For the ASN, the following relationship is defined:

Table 11.6 Comparison of reference plan acceptance probabilities (P_{ar}) and skip-lot sampling plan acceptance probabilities (P_{asl}) for certain values of f and i

				Skip-lot sampling probability of acceptance (Pasl)								
				f and i = n			f and i = n/2			f and i = n/4		
p	n	c	Par	0.2	0.25	0.5	0.2	0.25	0.5	0.2	0.25	0.5
0.001	5	0	0.9950100	0.9989819	0.9987289	0.9974738	0.9989900	0.9987384	0.9974863	0.9989940	0.9987431	0.9974925
0.01	5	1	0.9990199	0.9998032	0.9997541	0.9995087	0.9998035	0.9997544	0.9995092	0.9998037	0.9997546	0.9995094
0.05	5	2	0.9988419	0.9997673	0.9997092	0.9994193	0.9997677	0.9997097	0.9994199	0.9997679	0.9997100	0.9994203
0.1	5	3	0. 9,995,400	0.9999078	0.9998848	0.9997697	0.9999079	0.9998849	0.9997698	0.9999079	0.9998849	0.9997699
0.001	10	0	0.9900449	0.9978448	0.9973198	0.9947736	0.9979281	0.9974167	0.9948980	0.9979607	0.9974548	0.9949477
0.01	10	1	0.9957338	0.9991172	0.9988989	0.9978213	0.9991321	0.9989163	0.9978441	0.9991380	0.9989232	0.9978532
0.05	10	2	0.9884964	0.9974789	0.9968674	0.9939158	0.9975910	0.9969975	0.9940819	0.9976347	0.9970486	0.9941484
0.1	10	3	0.9872048	0.9971671	0.9964824	0.9931910	0.9973066	0.9966443	0.9933965	0.9973610	0.9967076	0.9934788
0.001	20	0	0.9801889	0.9946176	0.9934194	0.9881384	0.9953654	0.9942675	0.9891067	0.9957111	0.9946664	0.9895993
0.01	20	1	0.9831407	0.9956174	0.9946225	0.9901507	0.9961460	0.9952252	0.9908554	0.9963929	0.9955108	0.9912122
0.05	20	2	0.9245163	0.9588070	0.9535293	0.9375192	0.9732778	0.9681311	0.9481636	0.9796082	0.9750571	0.9549464
0.1	20	3	0.8670467	0.8919629	0.8866524	0.8742944	0.9321830	0.9227175	0.8927898	0.9550845	0.9461739	0.9107707
0.001	40	0	0.9607702	0.9782894	0.9755609	0.9673557	0.9859723	0.9832883	0.9729291	0.9893419	0.9869693	0.9765117
0.01	40	1	0.9392634	0.9542044	0.9512034	0.9438437	0.9716498	0.9672893	0.9527560	0.9806426	0.9766687	0.9604169
0.05	40	2	0.6767358	0.6767360	0.6767359	0.6767358	0.6772597	0.6771289	0.6768669	0.7008432	0.6951598	0.6831197
0.1	40	3	0.4231307	0.4231307	0.4231307	0.4231307	0.4231307	0.4231307	0.4231307	0.4235548	0.4234489	0.4232368
0.001	60	0	0.9417363	0.9474675	0.9461430	0.9432832	0.9649141	0.9610393	0.9499947	0.9778089	0.9737451	0.9585720
0.01	60	1	0.8787667	0.8789744	0.8789225	0.8788187	0.8880419	0.8858588	0.8812267	0.9230586	0.9153247	0.8940189
0.05	60	2	0.4174358	0.4174358	0.4174358	0.4174358	0.4174358	0.4174358	0.4174358	0.4174405	0.4174393	0.4174370
0.1	60	3	0.1373986	0.1373986	0.1373986	0.1373986	0.1373986	0.1373986	0.1373986	0.1373986	0.1373986	0.1373986

$$\mathrm{ASN}\left(\text{skip-lot}\right) = \mathrm{ASN}\left(\mathrm{R}\right)^{*} f \tag{11.2}$$

Where ASN(R) is the average sample number for the reference sampling plan and f is the fraction of total lots that are inspected. For a single sampling plan of reference, ASN(R) is given as 'n', then Eq. 11.2 becomes:

$$\mathrm{ASN}\left(\text{skip-lot}\right) = n^{*} f \tag{11.3}$$

For ARL, there are two different average run lengths to consider: ARL-1 and ARL-2. ARL-1 is the average number of lots to the occurrence of a rejection of a process quality shift from p_0 (original percent defective) to p_1 (new percent defective) (where $p_0 < p_1$) where the count is over all of the lots. ARL-2 is the average number of lots to the occurrence of a rejection following the prior rejection. These two quantities are defined below:

$$ARL-1 = \left(\frac{1}{1-p_1}\right) + \left[\left(\frac{\left(1-f\right)\left[p_0^i + f\left(1-p_0\right)\sum_{j=0}^{i-1} p_0^j p_1^{i-j}\right]}{f\left(1-p_1\right)\left[f+\left(1-f\right)p_0^i\right]}\right)\right]$$

and (11.4)

$$ARL-2 = \left[\frac{1}{1-p_{asl}\left(f,i\right)}\right]$$

The AOQL of a sampling plan is the maximum value on the AOQ curve. It is applicable for defective units, defects per unit, and defects per quantity. It is expressed as either a defective rate (fraction defective, percent defective, dpm) or as a defect rate (defects per unit, defects per 100 units, dpm). The AOQ curve gives the average outgoing quality (left axis) as a function of the incoming quality (bottom axis). The AOQL is the maximum or worst possible defective or defect rate for the average outgoing quality. Regardless of the incoming quality, the defective or defect rate going to the customer should be no greater than the AOQL over an extended period of time. Individual lots might be worse than the AOQL but longer term, the quality should not be worse than the AOQL. The AOQ curve and AOQL assume rejected lots are 100% inspected, and is only applicable to this situation. Therefore, for our purposes we will not examine this property for skip-lot sampling plans. Further information on skip-lot sampling is given through an example in Sect. 11.8.4.

11.7 Considerations in the Implementation of Stringent Sampling Plans

11.7.1 Conceivable Alternative Plans

As indicated above, for foodborne pathogens such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer;

2. There is the practical advantage of combining (e.g. pooling) many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome;
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may perhaps not be contaminated at all. But using the plan $n = 95$, $c = 1$ and having accepted one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

11.7.2 Erroneous Procedures for Stringent Sampling Plans

The two examples given below are in the context of erroneous applications of (or incorrectly applied) stringent sampling plans.

It might be suggested, for example, that if the plans $n = 60$, $c = 0$ and $n = 95$, $c = 1$ provide equivalent probability (for lots having 5% of units defective), an operator finding one positive in 60 analytical units might then proceed to examine another 35 (total $n = 95$) in the hope of clearing the lot if all the latter were negative. But such a procedure is in reality a two-stage plan $n_1 = 60$, $c_1 = 1$, plus $n_2 = 35$, $c_2 = 0$, which has a greater probability of accepting an unsatisfactory lot than $n = 95$, $c = 1$. In fact, this probability P_a is 0.07, compared with 0.05 for the one-stage plan. Although in this example the difference is not great, there are situations where such two-stage procedures can cause more serious error. In almost all cases, the probability of acceptance for two-stage plans will be greater than for one-stage plans, hence a note of caution in their use. Where two-stage sampling plans are actually being used, their OC curves should be computed and the resulting probabilities of acceptance evaluated.

A similar problem can arise where a plan requires a large number of analytical units, which would be unusually costly. Suppose the plan is $n = 95$, $c = 1$, but for economy a group of only 20 units is tested initially. If one unit should fail in this initial group, an analyst might examine the remainder (in this case 75) with the idea that if a defective is not found in the second group, the first may be ignored. Nevertheless, the sampling plan that has been applied actually corresponds to $n_1 = 20$, $c_1 = 1$ and $n_2 = 75$, $c_2 = 0$. No justification exists for 'preferring' the results from the second series. Similarly, if the plan were $n = 60$, $c = 0$ and an analyst felt uncertain about finding a defective unit in the first 20 samples and the lot was accepted on the basis of the remaining 40 samples being negative, then the actual plan applied would be the double sampling plan: $n_1 = 20$, $c_1 = 1$; $n_2 = 40$, $c_2 = 0$, which actually accepts more defective lots than would $n = 60$, $c = 0$. This is an illustration of errors in properly applying sampling procedures and evaluating the results.

11.8 Examples

11.8.1 Examples of the Moving Window

The 'moving windows' technique has been applied by the US Department of Agriculture Food Safety Inspection Service's Pathogen Reduction/HACCP regulation for testing carcasses and ground meat for *Salmonella* and poultry carcasses, and other products derived from these carcasses for *Campylobacter.* A window consists of a certain number of analyses starting from the most recent. As

each new test result is obtained the window moves up with the oldest result being discarded. Performance is determined by the number of positives (either presence or absence if by an attribute plan or a limit if a variables plan). In the case of *E. coli* testing, the technique was adapted for variables testing (i.e., considering quantitative data) using a limit that could not be exceeded (*M* value) and a warning value (*m* value) that could not be exceeded more than 3 times (*c* value) in a moving window of 13 tests ($n = 13$). The *m* value and *M* were based on national baseline surveys of meat and poultry carcasses and ground meats and are specific for each animal species (USDA-FSIS 1996). The rate of sampling is also commodity specific; for example for beef, the values for generic *E. coli* were set at $m = 14$, $M = 100$.

The method for evaluating carcasses for *Salmonella* consists of presence/absence testing. Performance at each facility is measured through a series of samples referred to as a set of size *n*. When sampling with two possible outcomes (either positive or negative), the number of positives in independent samples was assumed to have a binomial distribution. USDA-FSIS decided that a facility operating at the baseline prevalence for *Salmonella* in carcass or ground meat samples should have an 80% probability of passing. This figure was set to balance the need to prevent an establishment from failing based on chance and also the need to identify establishments likely to be operating above the prevalence. A sampling plan or performance is then designed, based on baseline prevalence, in a similar manner to that described earlier in this chapter (Legan et al. 2000) to give each establishment an 80% probability of passing.

As described, the *Salmonella* performance standards are not lot acceptance/rejection standards. The detection of *Salmonella* in a specific lot of carcasses or ground product does not, by itself, result in condemnation of the lot. Instead, the standards are intended to ensure that each establishment is consistently achieving an acceptable level of performance with regard to controlling and reducing enteric pathogens on raw meat and poultry products (USDA-FSIS 1996). In 2015, the FSIS introduced routine sampling of raw chicken parts as one of the several routine verification testing programs. The moving window approach was introduced rather than the consecutive day approach for assessing all verification testing (FSIS 2015).

An example of the moving window approach applied in the EU (EFSA 2010) is shown in Fig. 11.2. In this case, sampling is carried out 1 day of the week on a weekly basis. At each sampling point, neck skins from broiler carcasses are collected and pooled in groups of 3, leading to 5 pooled samples with a weight of 25 g per pool. Results are recorded over a rolling period of 10 weeks and the criteria are $n = 50$, $c = 5$, m = absence of *Salmonella* in 25 g. Compliance can be met only when stable and appropriate *Salmonella* levels are recorded. In the example shown, a *Salmonella* problem is detected between weeks 8 to 11, leading to non-compliance with the criteria from week 11 to 17. The stringency of this sampling plan increased in 2011 when prior to this, $c = 7$ was applied. Through this sampling procedure, food producers are able to react following a lower number of repeated positive results compared to considering only the total number of positives in 10 weeks.

The New Zealand Food Safety Authority (NZ FSA) has also used the moving window technique in their risk management strategy for control of *Campylobacter* in poultry. Microbiological monitoring of meat and poultry produced in New Zealand generates data for the National Microbiological Database that was developed in response to the emerging international HACCP environment and the need to develop 'equivalence' of food safety controls in different countries, with the primary purpose of underpinning development of performance criteria. In this particular case, samples were taken from every flock coming in for slaughter. For processing operations which slaughtered more than one million broilers per year, three rinsate samples were taken per processing day (random) for 5 days, and 45 samples taken over 15 processing days, representing the moving window, equalling 3 processing periods. The performance target is applied to the 3 processing periods. Addition of sample results for the next processing period displaced the results from the oldest processing period. In this case, the "moving window limit" failure occurs when the log count for 7 or more out of the 45 samples from the moving window are greater than the target of 3.78 $\log_{10}$ cfu/carcass. For processing operations

	Week number																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total positive pools after 10 weeks
Number of positive pools per week (out of 5)	0	0	0	0	0	0	0	2	2	1											5
		0	0	0	0	0	0	2	2	1	1										**6**
			0	0	0	0	0	2	2	1	1	0									**6**
				0	0	0	0	2	2	1	1	0	0								**6**
					0	0	0	2	2	1	1	0	0	0							**6**
						0	0	2	2	1	1	0	0	0	0						**6**
							0	2	2	1	1	0	0	0	0	0					**6**
								2	2	1	1	0	0	0	0	0	0				**6**
									2	1	1	0	0	0	0	0	0	0			5
										1	1	0	0	0	0	0	0	0	0		2
											1	0	0	0	0	0	0	0	0	0	1

Fig. 11.2 An example of the 'moving window' sampling scheme for *Salmonella* in broiler carcasses (results of sampling over 10 consecutive weeks), with positive samples concentrated in a short period, adapted from EFSA (2010). Bold numbers indicate non-compliance

slaughtering less than one million broilers per year, 3 samples from 1 day each processing period were taken resulting in 9 samples per moving window. Failure occurred when the log count for 2 or more out of 9 samples from the moving window (three successive processing periods) were greater than the target of 3.78 $\log_{10}$ cfu/carcass.

11.8.2 An Example of Reduced Sampling: Sampling in Slaughterhouses and at Premises Producing Minced Meat and Meat Preparations in Europe

In the case of sampling for *Salmonella* analyses of minced meat, meat preparations and carcasses, European legislation (EU No. 2073/2005, amended in EC No. 1441/2007) requires that food business operators of slaughterhouses or establishments producing minced meat, meat preparations or mechanically separated meat shall take samples for microbiological analysis at least once a week. However, this frequency can be reduced to every 2 weeks if satisfactory results have been obtained for 30 consecutive weeks. The *Salmonella* sampling frequency may also be reduced if there is a national or regional *Salmonella* control program in place and if this program includes testing that replaces the described sampling. The sampling frequency may be further reduced if the national or regional *Salmonella* control program demonstrates that *Salmonella* prevalence is low in animals purchased by the slaughterhouse. Regarding sampling of minced meat and meat preparations for *E. coli* and aerobic colony count analyses and the sampling of carcasses for Enterobacteriaceae and aerobic colony count analyses, the frequency may be reduced to fortnightly testing if satisfactory results are obtained for 6 consecutive weeks.

11.8.3 An Example of Tightened Sampling: Aseptically-Packed Products

Normal sampling of aseptically filled products is commonly elevated to a higher level following particular events. These events include failures in packs from cases packed directly after start-up or post-resterilization, after intervention stoppage with an associated failure in a subsequent pallet sample, if there is more than one failure in the same production run or if any failure is confirmed through resampling and retesting. Other exceptional circumstances that will trigger an escalation in sampling include: failure to reliably meet seal parameters; filling problems (low/high weight); seal integrity issues; steam barrier failures; product held in aseptic tank for long periods of time. For a new aseptic line, an intensive sampling regime is required to demonstrate sterility and the target defective rate for these processes is normally less than or equal to 1 in 10,000. For sterility testing in a newly installed line, a liquid microbiological medium (broth) is commonly used to demonstrate the line is running at this target defect rate (95% confidence).

As an example, the initial sample size may be of the order of 30,000 packs, with at least 3 runs of 10,000 packs with cleaning in between runs (Fig. 11.3). Broth is used to facilitate quicker assessment, and to allow growth of a broad range of microbiological contaminants. To detect failures, pH reduction (by visual inspection for a color change) or detection of microbial biomass (by visual inspection for turbidity/opaqueness) may be used. If a successful outcome (no defects) is achieved, testing can proceed to product trials. If the outcome is unsuccessful (1 or more defects), the cause of the defect(s) is investigated, to rectify problems and the broth test is repeated. When 30,000 units are examined and 1 or more defects are found, it is possible to estimate the actual defective rate (with 95% confidence) (Fig. 11.4). It may be advisable to run a much smaller broth test prior to this performance broth test to ensure there are no major insterility problems.

The main purpose of these product trials is to determine any adverse effects of the product formulation (i.e., viscosity and particulate load) and related key operational activities (e.g., start-up, line stoppages) on the commercial sterility of the product. The statistical objective is to demonstrate that there is no significant difference between food and microbiologial medium (i.e., the defect rate is still ≤ 1 in 10,000). A sample size of 3000 will enable the detection (95% confidence) of a marked increase in the defective rate (e.g., 3000 samples give 25% probability to detect 1/10000 and 95% to detect 1/1000).. A smaller sample size has less chance of detecting the same increase (e.g. a sample size of 500 has

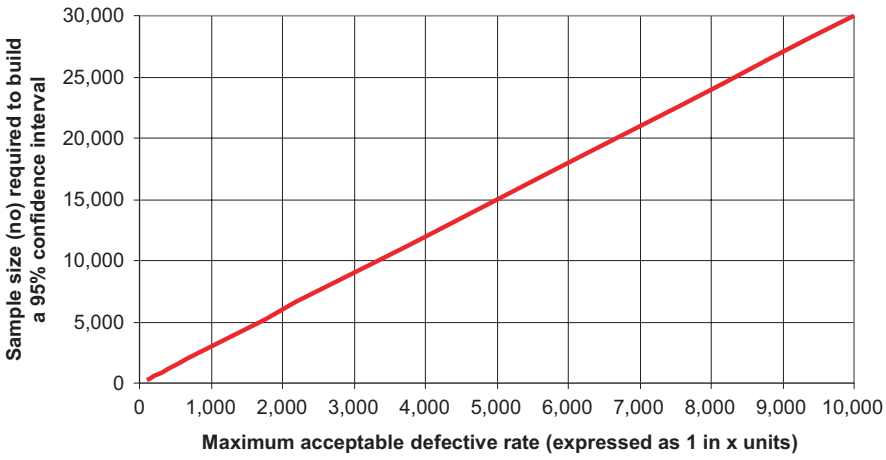

Fig. 11.3 Sample size required to demonstrate that the defective rate is less or equal to the maximum chosen, when 0 defectives are found

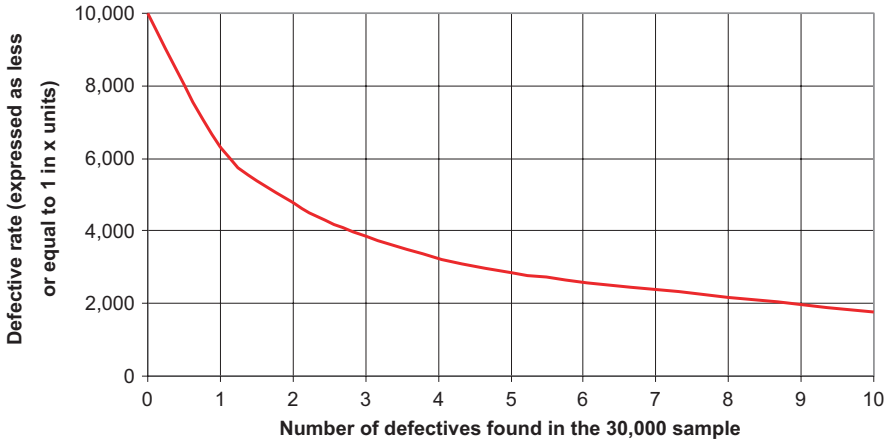
Defective rate (expressed as less or equal to 1 in x units)
10,000
8,000
6,000
4,000
2,000
0
0 1 2 3 4 5 6 7 8 9 10
Number of defectives found in the 30,000 sample

Fig. 11.4 Estimation of the defective rate (95% confidence) when 30,000 units are sampled and when 0, 1… 10 defectives are found

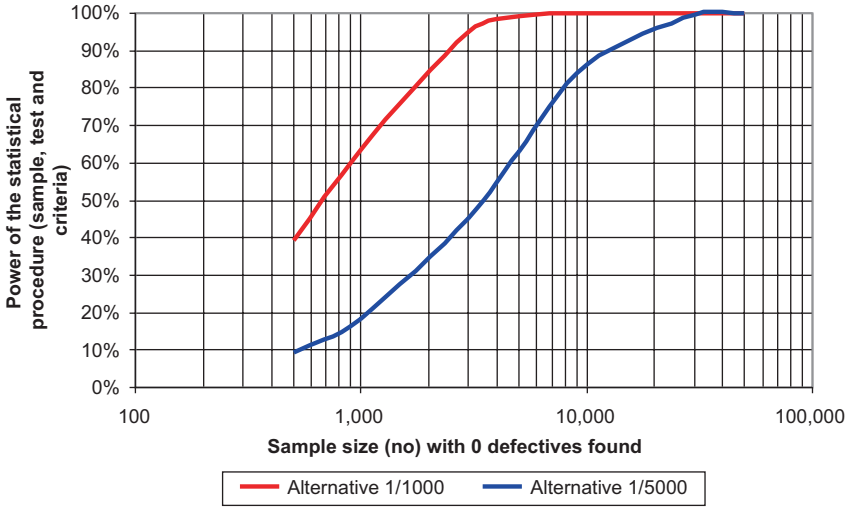

Fig. 11.5 Chance of detecting an increase in the defective rate (e.g. from 1 in 10,000 to 1 in 5000 or 1 in 1000) vs sample size

only a 40% chance), but, to have the same chance (95%) of detecting a small increase in defective rate, a much higher sample size would be required (e.g., a sample size of 20,000 to detect an increase to 1 in 5000) (Fig. 11.5). For this step, a 'worst-case' formulation, i.e., one most likely to adversely affect commercial sterility, is used. If the outcome is successful (number of defects = 0), commercial production can commence. Note, when 3000 units are analyzed and 0 failures are found, there is no marked increase in the defective rate. This reduced sample size does not re-assess the target defective rate, but provides evidence that it remains unchanged. With an unsuccessful outcome (1 or more defects), the cause should be investigated and the problem rectified.

11.8.4 Example of Reduced Sampling: Skip-Lot Sampling

In Sect. 11.6.1 the method of skip-lot sampling was described and the theory of the method provided. In this section, we will provide an example of skip lot sampling performed in the food industry. For this example the product is a container (called a "pot") of a dairy product with a sealed top. This product is aseptically packed on a filling line. For discussion purposes the containers are 10 oz., there are 12 containers in a tray (sometimes referred to as a case) and trays are then packed in pallets. In our example, we will consider one pallet to be the lot for sampling purposes, but the sampling is done based on a number of samples (n) that move through the packing line. From historical information we know that the typical defect rate is 1 in 10,000.

In this dairy product example, the sampling procedures will obviously be different for start-up, change over, and other similarly unique operations for this product. Therefore, the skip-lot sequencing described herein is for normal production runs, once the line is up and running and all unique procedures are completed. Figure 11.6 shows the schematic of the skip lot procedure; a written description follows.

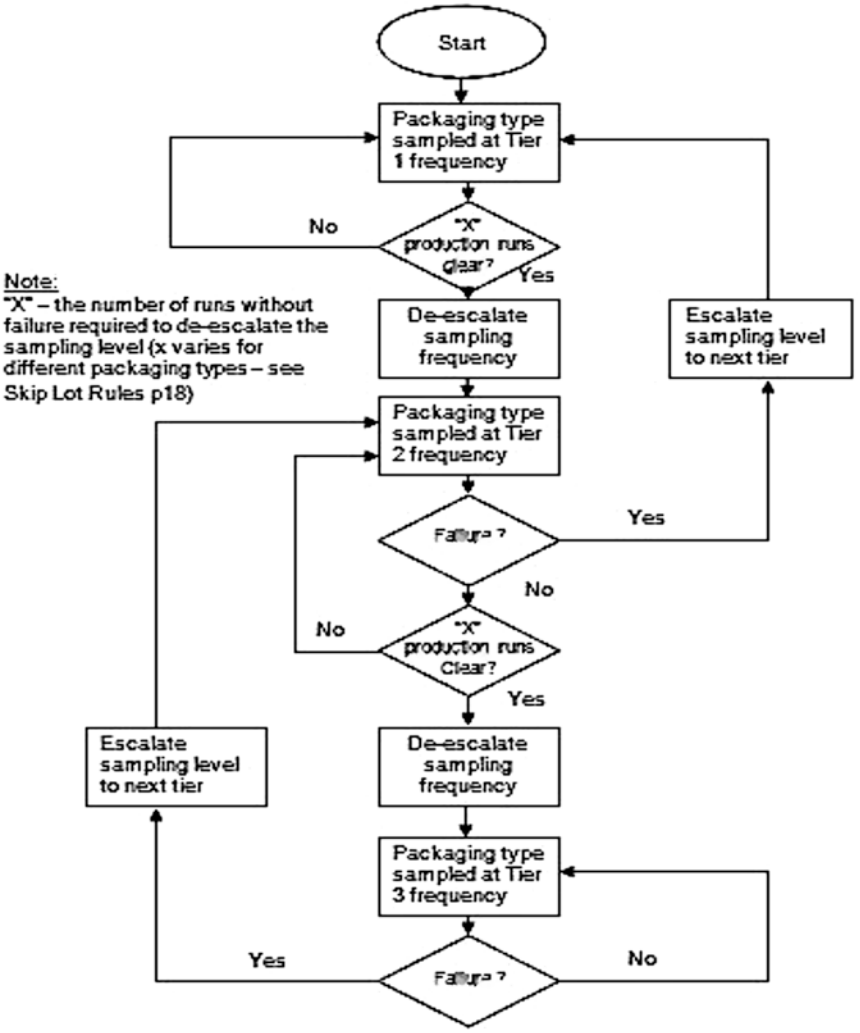

Fig. 11.6 A schematic diagram of the skip-lot procedure defined for a typical dairy product

Table 11.7 A comparison of a skip lot sampling plan for a dairy product where $n = 6$ and $c = 0$ and the defect rates of 1 in 10,000 and 1 in 100

Sampling method	# samples	Method	Prob acceptance (defect rate 1/10,000)	Prob acceptance (defect rate 1/100)
Tier 1 (reference plan)	$n = 468$	Every other pallet ($n = 468$)	P_{ar} ($n = 6$. $c = 0$) = 0.999994	P_{ar} ($n = 6$. $c = 0$) = 0.94148
Tier 2	$n = 702$	Every third pallet ($n = 702$)	P_{asl} ($f = 0.67$, $i = 468$) = 0.999996 (% difference = 0.0002)	P_{asl} (f = 0.67, $i = 468$) = 0.94148+ (% difference ~ 0.0)
Tier 3	$n = 936$	Every fourth pallet ($n = 936$)	P_{asl} ($f = 0.50$, $i = 702$) = 0.999997 (% difference = 0.0003)	P_{asl} ($f = 0.50$, $i = 702$) = 0.94148 (% difference ~ 0.0)

Once the unique procedures (start up, change over, etc) have been passed and the system is in the production running state, the normal sampling begins with a Tier 1 sampling plan (see Table 11.7). The Tier 1 sampling plan (called the reference sampling plan) will be followed until $n = 468$ pots are cleared. Once the clearance criteria have been met, the process will de-escalate to a Tier 2 sampling plan (a reduction in sampling) as seen in Table 11.7. In this skip-lot example a further reduction in sampling can be achieved once $n = 702$ (the Tier 2 clearance rate) have been cleared with no violations, thereby moving to a Tier 3 plan (see Table 11.7).

In this example, a two class sampling plan is used where the number of pots selected for sampling is 6 and the allowable defects in the sample is 0. This is representative of a two class plan where $n = 6$, $c = 0$; see Chap. 7 and Table 7.1 for further information on this type of sampling plan. In Sect. 11.6.1, the $c = 0$ class plan was noted as a cautious example because of the alpha and beta probabilities associated with this type of two-class plan. We show the example here.

As shown in Table 11.7, moving through the reduction in sampling (from Tier 1 to Tier 2 and then further to Tier 3), there is a slight increase in the probability of acceptance (P_a) of the lot (shown as percent (%) difference in the Table) and the new probability of acceptance is labeled as probability of acceptance under the skip-lot regime (P_{asl}). This slight increase in probability of acceptance (P_a) is to be expected; in the skip lot procedure we are skipping some samples and the assumption is that those samples are all acceptable samples. As can be seen in Table 11.7, the difference (increase) in P_a going from Tier 1 to Tier 2 is 0.0002% and from Tier 1 ultimately to Tier 3 is 0.0003%. These percentages are predictable and this follows as the defect rate is small (recall the defect rate in this aseptically packaged product is known to be about 1 in 10,000 samples). This increase in the P_a can be seen as an increase in risk or consumer's risk of accepting a product that should not be; albeit the risk is low in this example.

In this example, if at any time during sampling a lot violates the $n = 6$, $c = 0$ criteria, then sampling is escalated back to the previous Tier level (say from Tier 3 to Tier 2, etc). This rule is part of the process used in this particular example.

To further extend this same example and for a better understanding of the skip lot procedure, consider this change. Assume that the aseptic filling head did not get properly cleaned and the typical defect rate went from 1 in 10,000 samples to 1 in 100 samples (or a new 1% defect rate).

As is shown in Table 11.7, the change (increase) in P_a is negligible in this case. This small difference should be expected based on the theory of skip-lot sampling as given in Sect. 11.6.1. As the defect rate gets larger, then the probability of acceptance gets smaller (moves away from 1.0) and from Eq. 11.1 we can see that the P_{asl} (small defect rate, 1/10,000) > P_{asl} (large defect rate, 1/100) which follows from the theory.

Using this basic approach, other skip lot plans can be investigated for various defect levels as well as different types of sampling plans (shown in Chap. 7). The reader is cautioned to examine the results of their calculations carefully and to understand the significance of the increase in the prob-

ability of acceptance as an increase in consumer's risk (type II error) in accepting lots that are not acceptable. The balance to this risk comes from the reduced expense in sampling given by the skip-lot procedure.

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Chapter 12
Sampling to Assess Control of the Environment

12.1 Introduction

This chapter addresses the importance of microbiological testing to assess the effectiveness of control measures implemented to prevent product contamination from the environment. Preventing contamination of ready-to-eat foods is emphasized. While the discussion is limited to the verification of measures to control pathogens, the concepts can be applied to microbial spoilage. Routine environmental sampling is more frequently applied in food processing plants and less frequently at other steps along the food supply chain. Therefore, this chapter focuses on the verification of processing environment controls in facilities manufacturing foods since recontamination of food from equipment or other environmental sources is a significant contributing factor for foodborne disease (Reij et al. 2004). The role of environmental contamination in other parts of the food supply chain such as in primary production, during distribution, at retail and foodservice venues, and in consumer settings also exists. Microbiological sampling in these settings may be more problematic; however, potential applications are briefly discussed.

The microbiological safety of foods requires that effective control measures are implemented throughout the whole food supply chain. Good Agricultural Practices during primary production and harvesting of fruits, vegetables and animals are important to minimize the presence and levels of pathogens. Industrially manufactured foods require effective design and implementation of Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP) systems. GHP includes those prerequisite conditions that are necessary for pathogen control and implementation of an effective HACCP plan. Good practices during storage, distribution and further handling in subsequent steps in the food chain can minimize risks resulting from increases through growth or recontamination.

Knowledge of the microbial ecology of the environment is essential for many products. Several foodborne outbreaks associated with commercially manufactured foods reveal that weaknesses in GHP implementation can lead to post-process contamination with pathogens. Environmental contamination in the field has led to outbreaks associated with raw fruits and vegetables. Cross-contamination has also been associated with outbreaks at the retail and foodservice level.

In food safety management systems, prerequisite programs such as Good Agricultural Practices, Good Storage Practices and Good Hygiene Practices play an essential role in minimizing the presence of pathogens along the food chain. In HACCP systems, Critical Control Points (CCP) are applied to prevent, eliminate or reduce microbial hazards to acceptable levels. For many products, CCPs typically include a kill step (e.g., cooking, sterilization) whereas in minimally processed foods, CCPs

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may involve manipulation of one or more factors, such as low temperature storage, reduced a_w, reduced pH, etc., specifically designed to control growth of pathogens. Establishment of appropriate critical limits at specific processing steps helps to ensure safety of the products, taking into account possible fluctuations during operations.

Experience indicates that even optimal application of prerequisite programs or HACCP plans may not guarantee that contamination from the processing environment will not occur unless the product is in a sealed container (e.g., a canned product). Thus, it is possible to reduce, but not completely prevent or eliminate, the likelihood of contamination when food is exposed to the processing environment. When this potential is recognized, manufacturers may decide to establish procedures to implement zoning that identifies high hygiene areas and GHPs to minimize recontamination in these areas. Further, where environmental contamination can result in unsafe foods, establishment of a routine sampling program to assess control of the environment may be necessary.

12.2 Importance of Prerequisite Programs

The term "prerequisite programs" refer to those control measures that should be in place before implementation of a HACCP program. The basic elements of GHP for food processing are described in *General Principles of Food Hygiene* (CAC 2003). Some countries may establish their own standards; e.g., U.S. FDA's Current Good Manufacturing Practices (FDA 2015c). Numerous codes of hygienic practices for different products (e.g., canned foods, aseptically processed and packaged low-acid foods, spices and dried aromatic foods, infant formulae, frozen foods, fruits and vegetables) have been published by Codex Alimentarius and are available at http://www.codexalimentarius.org/standards/list-of-standards/.

The hygienic design of food manufacturing premises and processing equipment has been discussed, e.g., Holah and Lelieveld (2011), Baker (2013) and Fortin (2011), and product specific documents published by international organizations are also available, e.g., the meat industry (FAO/FIC 2004), chilled foods (ECFF 2006), dairy products (Anonymous 2010b), bottled water (EFBW 2012), eggs (AECL 2010) and nuts (GMA 2010).

Some of the more important elements of prerequisite programs that can minimize contamination from the processing environment are:

- Layout of processing lines and control of the movement of personnel and mobile equipment to minimize cross contamination from raw materials to finished product,
- Equipment design and location for cleanability,
- Appropriate cleaning and disinfecting procedures that are targeted toward the pathogens of concern for the particular food and process, including minimizing the potential spread of pathogens through inappropriate use of high pressure air or water,
- Scheduled preventive maintenance to minimize breakdowns during operation,
- Appropriate waste removal, and
- Training and behavior of personnel relative to the target pathogens

Visual and other sensory inspections are valuable to assess adherence to and effectiveness of GHPs. While such inspections can be performed by trained specialists, all operators should be trained to be constantly vigilant and to report deviations from normality. Microbiological surveillance for pathogens or appropriate indicators provides supporting information to assess whether the environment is under control.

The potential for contamination should be considered in the design and implementation of GHP programs and if contamination is likely to occur, some means for assessing its effectiveness should be

adopted. Each manufacturing facility must consider control measures that are appropriate for the conditions of operation, the food, the process and the pathogens of concern to minimize in-process contamination (Tompkin 2002; Cordier 2008; Jones 2011; Ball et al. 2011), and implement root cause investigations when problems occur (Kornacki 2010).

Numerous guidelines and regulations have been published to help improve hygiene and food safety in the food supply chain. Examples for primary production are Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption (FDA 2015a); Guide for Good Farming Practices (FAO/IDF 2011); the Guidelines for Good Agricultural Practices (FAO/Embrapa 2002); and documents specific to individual commodities such as pumpkin (IICA 2006), lettuce and leafy greens (Anonymous 2006; FDA 2009), mushrooms (Anonymous 2010a) or nuts with addenda for pecans and almonds (GMA 2010). FAO also maintains a database with more than 800 documents related to good agricultural practices (http://www.fao.org/prods/GAP/home/database_en.htm).

Guidelines for other parts of the food supply chain are also available, including Guidelines in Good Hygiene Practices for Vending and Dispensing (AVA 2008), for retail (Kozak et al. 2014) and food service operations (NSW Authority 2007; BRC 2009; FDA 2013), and wholesale distributors (FSA 2007).

12.3 Microbial Contamination from the Environment

12.3.1 Contamination During Primary Production

As previously discussed, general and commodity specific guidelines and regulations exist to reduce microbial contamination during primary production. The application of these prerequisite programs must consider the nature of the product, production practices, potential hazards and other factors, thus it is beyond the scope of this chapter to address microbial contamination for all commodities. Control of contamination during primary production is relevant for commodities that are generally consumed in the raw state, such as many fruits and vegetables, bivalve mollusks, certain fish (e.g., for sushi), milk for raw consumption, etc. More information on potential contamination sources during primary production is available (ICMSF 2005, 2011).

To illustrate general concepts, prerequisite program considerations for produce items that are consumed raw are used as an example. These focus on elements such as:

- Water resources and management appropriate to the crop,
- Hygiene of equipment, tools, and buildings,
- Management of pests and other animal intrusion appropriate to the crop,
- Worker training and health, including hygiene, and waste management in the field, and
- Biological soil amendment management, including manure use

Several outbreaks attributed to raw commodities have been investigated to identify the routes of contamination. In numerous cases, contamination was demonstrated to occur in the field during primary production. In commodities such as tomato, jalapeño pepper, spinach and other leafy greens, and berries, there are multiple routes of contamination including the application of organic wastes as fertilizers to fields used to grow crops (Strawn et al. 2011, 2013); contamination of waters used for irrigation or application of pesticides/herbicides with fecal material (Pachepsky et al. 2011); direct contamination by livestock, wild animals and birds (Jay-Russell 2013); as well as post-harvest contamination through workers (Waitt et al. 2013).

12.3.2 Environmental Contamination in Processing Facilities

Foods cooked in the container in which they are sold and foods packaged using aseptic packaging systems are protected from contamination. Foods that are pasteurized, cooked, or subject to other processes that reduce pathogens may be contaminated when they are exposed to the environment before filling or packaging. Manufacturers of such foods must take every reasonable precaution to prevent contamination after the food is cooked and before it is packaged, but it is impossible to prevent it completely.

Some ready-to-eat foods are not cooked, but receive mild treatments that may alter their microbiota. These foods may acquire microbiota from the processing environment. For example, a range of fish products (e.g., pickled, gravid, cold-smoked) receive very mild treatments, and they are likely to carry microbiota that reflect the microorganisms associated with the post-processing equipment and environment.

Ready-to-eat foods are subjected to a wide range of processing conditions, some of which are intended to eliminate pathogens. Subsequent handling, storing, conveying, sorting and packaging, etc. create opportunities for contamination. The environment to which the foods are exposed can be a potential source of pathogens. Pathogens such as *Salmonella* or *Listeria monocytogenes*, and also spore-formers, can be introduced into a food operation by a number of vehicles. Conditions permitting, they may become established and multiply, particularly in sites in the processing environment that are difficult to clean and disinfect. If this occurs during production, the pathogens may be transferred to product contact surfaces in the vicinity of the site and pass downstream with the flow of the product. Evidence indicates that some pathogens, e.g., *L. monocytogenes*, can become established in the processing environment and persist for long periods, even years.

The probability of contamination of the food product increases with prevalence. The concentration of the pathogen is influenced by the type of process, hygiene procedures and whether those procedures can spread the pathogen if they are not properly performed. Severe failure of one or more steps in GHP may lead to high levels of pathogens in the processing environment and inevitably lead to product contamination. Adequate procedures to prevent establishment of sites or niches supporting microbial growth and survival in the processing environment are essential to prevent subsequent product contamination, especially in ready-to-eat products.

12.3.2.1 Examples of Outbreaks Due to Recontamination from the Processing Environment

Numerous foodborne illness outbreaks associated with the consumption of processed foods that were re-contaminated from the environment have been reported (Table 12.1). While the number of outbreak-associated foods from industrial establishments is relatively small in comparison to foods prepared in foodservice operations, catering, or other settings (Todd et al. 2007a; OzFoodNet Working Group 2012; CDC 2013; EFSA 2014), the size of the outbreaks associated with industrially manufactured foods can be very large due to the quantity of product distributed throughout a country or region. In addition, some outbreaks last several months or even years, before they are recognized and the source of the manufactured food is identified. Outbreak investigation data are available to demonstrate the importance of post-process contamination in many outbreaks.

Dehydrated dairy products were one of the first product categories associated with *Salmonella* post-process contamination. In 1977, several salmonellosis outbreaks associated with dairy-based powdered products led to investigation in several Australian plants and revealed the occurrence of salmonellae in processing environments as well as parts of equipment such as the insulation of a drying tower. The outcome of the investigations stimulated the improvements in the construction and management of dairy factories manufacturing dehydrated products (Forsythe et al. 2003).

Table 12.1 Examples of outbreaks attributed to environmental contamination

Product	Pathogen	Comments	Reference
Canned salmon	*C. botulinum*	Contamination from the processing environment, cooling water	Anonymous (1984); Stersky et al. (1980)
Ice cream	*E. coli* O145:H28 and O26:H11	Contamination from the processing environment	Buvens et al. (2011)
Different foods	*E. coli* O157:H7	Contaminated meat grinder and equipment at retail level	Banatvala et al. (1996)
Flavored yogurt	*E. coli* O157:H7	Pump previously used for raw milk	Morgan et al. (1993)
Pasteurized milk	*E. coli* O157:H7	Contamination from bottling-machine pipes and seals	Upton and Coia (1994)
Butter	*L. monocytogenes*	Contamination from the processing environment	Lyytikainen et al. (2000)
Cantaloupe	*L. monocytogenes*	Contamination from the processing environment	McCollum et al. (2013)
Celery	*L. monocytogenes*	Contamination from the processing environment	Gaul et al. (2013)
Cheese	*L. monocytogenes*	Contamination from the processing environment	Linnan et al. (1988)
Cheese	*L. monocytogenes*	Contamination from the processing environment and during distribution at retail	Gaulin et al. (2012)
Cheese	*L. monocytogenes*	Contamination from the processing environment	Koch et al. (2010)
Deli meats	*L. monocytogenes*	Contamination from the processing environment	Currie et al. (2015)
Hot dogs	*L. monocytogenes*	Contamination from the processing environment	Anonymous (1999)
Ice cream	*L. monocytogenes*	Contamination from the processing environment	FDA (2015b)
Water	Norovirus	Sewage water leakage into drinking water pipe	Van Alphen et al. (2012)
Breakfast cereals	*Sal.* Agona	Contamination from the processing environment	Russo et al. (2013)
Cooked meat	*Sal.* Agona	Contamination from the processing environment	Nicolay et al. (2011)
Soft cheese	*Sal.* Berta	Cheese ripening in buckets previously used for chicken carcasses	Ellis et al. (1998)
Infant formulae	*Sal.* Ealing	Contamination from the processing environment, insulation material of the drying tower	Rowe et al. (1987)
Chocolate	*Sal.* Eastbourne	Contamination from the processing environment	Craven et al. (1975)
Ice cream	*Sal.* Enteritidis	Pasteurized ice cream mix in tanker truck previously used for transporting raw liquid eggs	Hennessy et al. (1996)
Pastry	*Sal.* Enteritidis PT4	Equipment previously used for raw eggs or insufficiently cleaned piping and nozzles used for cream	Evans et al. (1996)
Salami	*Sal.* Montevideo	Contamination from the processing environment	Lienau et al. (2011)
Yeasts	*Sal.* München	Contamination from the processing environment	Joseph et al. (1991)
Chocolate	*Sal.* Napoli	Possibly contaminated water used in double walled pipes, tanks and other equipment	Gill et al. (1983)
Pet food	*Sal.* Schwarzengrund	Contamination from the processing environment	Behravesh et al. (2010)
Peanut butter	*Sal.* Tennessee	Contamination from the processing environment	Sheth et al. (2011)
Salmon	*Sal.* Thompson	Contamination from the processing environment	Friesema et al. (2014)
Canned meat	*Sal.* Typhi	Use of non-potable water for can cooling	Ash et al. (1964); Stersky et al. (1980)
Cooked sliced ham	*Sal.* Typhimurium	Cooked ham placed into containers previously used for curing raw pork	Llewellyn et al. (1998)
Pasteurized milk	*Sal.* Typhimurium	Possibly cross-connection between raw and pasteurized milk	Lecos (1986)
Peanut butter	*Sal.* Typhimurium	Contamination from the processing environment	Cavallaro et al. (2011)
Lasagna	*Sta. aureus*	Growth of S. aureus in the processing equipment, improper cleaning	Woolaway et al. (1986), Aureli et al. (1987)
Chocolate milk	*Y. enterocolitica*	Probably during manual mixing of pasteurized milk and chocolate syrup	Black et al. (1978)

An outbreak of *Salmonella enterica* serotype Agona infections associated with breakfast cereal was identified in April 2008 (Russo et al. 2013). *Salmonella* Agona strains that were indistinguishable from the outbreak strain were isolated from 2 of 17 cereal samples collected from case patient homes. In addition, the outbreak strain was found in 12 environmental samples and 9 product samples collected during inspection of the cereal production facility. The same production facility was implicated in a *S.* Agona outbreak in 1998 with the same outbreak strain (Breuer 1999). Construction activities at this facility seem to have allowed reintroduction of *S.* Agona into the processing environment, ultimately leading to contamination of product. This example highlights the persistence of *Salmonella* in dry food production environments and the impact it may have.

The investigation of a *Salmonella* Typhimurium outbreak occurring between September 2008 and April 2009 that caused more than 700 illnesses established a link to contaminated peanut butter and peanut-containing products. Investigations at the site manufacturing the peanut butter revealed poor manufacturing and hygienic practices, including evidence of rain and water leakage into storage areas, which can contribute to multiplication; potentially inappropriate roasting conditions, which can result in insufficient killing; and insufficient separation of areas handling raw and roasted peanuts, which can contribute to post-process contamination. Similar issues were identified in a second plant owned by the manufacturer, thus magnifying the problem (Cavallaro et al. 2011).

In 2006, the Pennsylvania Department of Health identified a small cluster of *Salmonella* Schwarzengrund infections, without identifying the source. New cases reappeared in May 2007 and exposure to dogs or dry dog food was suggested, but no brands made by a single plant could be identified. In July 2007, pet food contaminated with *S.* Schwarzengrund was detected by the FDA and 1 out of 144 environmental swabs obtained from the plant manufacturing the product yielded the outbreak strain. The positive swab was from the enrobing/flavoring room located after the kill step. More than 23,000 tons of pet food were recalled and the manufacturer suspended operations to renovate the plant and conduct sanitization before resuming production. Additional cases reported in May 2008 prompted a new recall of 105 brands of pet food and the definitive closure of the plant (Behravesh et al. 2010).

Contamination of food products with *L. monocytogenes* during processing has been documented for numerous outbreaks involving refrigerated, ready-to-eat products that support growth of this pathogen (see Table 12.1). Persistence of this pathogen over prolonged periods of time in the processing environment is an important factor contributing to recurrent issues. The effectiveness of hygiene control measures implemented in processing sites, such as layout of processing lines, design of premises and equipment, cleaning and sanitization procedures, as well as personnel awareness and training are key elements in the prevention of contamination. Factors related to the persistence of *L. monocytogenes* in processing environments as well as their impact on contamination and associated public health and economic issues were reviewed (Anonymous 2008; Ferreira et al. 2014).

12.3.3 Contamination During Distribution, Retail and at the Consumer Level

Contamination in steps in the food supply chain after processing (e.g., during distribution, retailing, preparation and handling in foodservice operations or at home) also occurs. Reviews have been published by Carrasco et al. (2012) and Farber and Forsythe (2014) and many case studies are available, including those that demonstrate that contamination is not a new phenomenon, e.g., Salvat et al. (1995). Pathogens in the environment in which food is handled and prepared can originate from infected food workers, raw foods, or other environmental sources. The most frequent source of contamination of food by handlers is the fecal-oral route, but other modes of transmission such as contact with contaminated raw foods, aerosols (sneezes), vomitus and infected lesions have been linked to outbreaks. Todd et al. (2007b, 2008) reviewed the role of food workers in 816 outbreaks.

12.4 Establishment and Growth of Foodborne Pathogens in the Processing Environment

Effective GHP procedures targeted toward the pathogens of concern require knowledge of the incidence, distribution, fate and behavior of the pathogens in food processing environments. A fundamental question that must be considered for each manufacturing facility is whether contamination is due to transient or persistent/resident pathogenic microorganisms.

12.4.1 Transient Versus Established Microorganisms

Understanding the difference between transient and resident microorganisms is fundamental to designing a system to control in-process contamination. This distinction is essential when investigating and correcting a contamination problem (see Sect. 12.6.8 and Chap. 11). A determination of whether pathogens are transient or resident can be made using a variety of traditional or more sophisticated DNA-based typing methods. Recovering the same isolates over a period of time is evidence that the pathogen is a resident of the environment. General reviews on the persistence of foodborne pathogens in processing environments have been published by Podolak et al. (2010) and Beuchat et al. (2011, 2013) for low moisture foods and Larsen et al. (2014) for *L. monocytogenes*, *Salmonella* and *Cronobacter* in primary and secondary production.

Transient microorganisms are introduced into a food plant through raw materials, humans, packaging supplies, etc., and do not typically become established in the environment. Transients, however, can contribute significantly to the types and numbers of microorganisms on the resulting food. Each raw agricultural commodity carries a wide variety of microorganisms and, possibly, certain pathogens (ICMSF 1988, 2011). In general, industrial operations convert raw commodities into further processed products under conditions that minimize cross-contamination during processing. Cleaning and disinfecting procedures normally are adequate to control the transient flora so that each day of operation is separate from the previous day. For example, salmonellae serotypes present in one group on animals on day 1 may differ from those on day 2, and the resulting raw meat or poultry from each day will reflect the difference. Upon adequate cooking, the salmonellae are killed and, should the products become contaminated, the serotypes will reflect those in the post-cooking environment. This concept applies generally to most commodities that receive a kill step during processing.

Resident microorganisms are introduced into the environment, become established, multiply and persist for days or years. Normal cleaning and disinfecting procedures control their numbers but may not eliminate them from the environment, considering that most floors, walls, overheads are not designed to be cleaned with the same efficacy as direct product contact surfaces. Commercial experience suggests that pathogens can be categorized as follows:

- Extensive history of establishment – non-typhoidal *Salmonella*, *Cronobacter* spp. and *L. monocytogenes*,
- Limited history, but potential for establishment exists – *Staphylococcus aureus, E. coli O157:H7*, spore-formers, and
- No history of establishment – *Yersinia enterocolitica, Salmonella* Typhi, *Shigella, Campylobacter* spp., viruses and parasites

12.4.1.1 Extensive History of Establishment – *Salmonella, Cronobacter L. monocytogenes*

Jakočiūne et al. (2014) investigated whether recurrent contamination of pasteurized egg products from a large European manufacturer was caused by a persistent strain of *Salmonella* Tennessee. Strains collected over a 3- year period were compared with pulsed-field gel electrophoresis (PFGE) and 45 out of the 73 isolates were shown to belong to one profile type. It was concluded that the contamination was caused by a persistent strain that had apparently adapted to grow in the egg products.

The persistence of different *Salmonella* species in feed mills and feed processing environments has been investigated by several authors in different parts of the world (Vestby et al. 2009; Davies and Wales 2013; Habimana et al. 2014; Pellegrini et al. 2015). *Salmonella* is capable of growing and surviving in numerous types of environments. They are exposed to many stresses such as nutrient limitation/starvation, acid/base, high/low temperatures, high/low osmolarity or desiccation. This exposure generates specific physiological responses, triggering resistance that contributes to their survival. These stress responses and survival capabilities can have a profound impact on the epidemiology and pathogenesis of this pathogen (Spector and Kenyon 2012).

Several studies have investigated the distribution of *Cronobacter* spp. in milk powder and infant formula producing plants. Reservoirs representing potential sources of contamination over time have been identified (Cordier 2008; Mullane et al. 2008; Craven et al. 2010; Reich et al. 2010; Jacobs et al. 2011).

The persistence of *L. monocytogenes* strains in processing environments has been demonstrated for several types of ready-to-eat foods. Lundén et al. (2003) found persistent and non-persistent sites in the meat and poultry plants, showing that the proportion of persistent PFGE types in the heated products was 8 times higher than in raw products, showing the importance of those strains in the post-process contamination. Blatter et al. (2010) investigated the processing environment of a sandwich production site. Persistence of a particular genotype of *L. monocytogenes* in the environment (including equipment such as slicers and conveyor belts) for more than 9 months was demonstrated, before they could be eradicated through revised cleaning and disinfection procedures.

Cantaloupe was the source of a multistate outbreak of listeriosis. Fruits and environmental samples from washing and drying equipment collected during the investigation yielded isolates matching all five outbreak-related subtypes, confirming that whole cantaloupe produced by the firm was the outbreak source (McCollum et al. 2013). Similar observations were made for many other foods, including cheese (Fox et al. 2011; Rückerl et al. 2014; McIntyre et al. 2015), mussels (Cruz and Fletcher 2011), fish and in particular smoked salmon (Holch et al. 2013; Rotariu et al. 2014), meats (Martín et al. 2014), egg products (Rivoal et al. 2013) and frozen and fresh-cut vegetables (Ballesteros et al. 2011; Lehto et al. 2013).

12.4.1.2 Limited History But Potential for Establishment Exists – S. *aureus*, *E. coli* O157:H7, Sporeformers

None of the staphylococcal enterotoxin outbreaks summarized by Hennekinne et al. (2012) were associated with the occurrence of persistent strains in processing environments. Bennett et al. (2013) indicated that cross-contamination in the food processing and preparation environment occurred in 13% of the outbreaks due to *Bacillus cereus*, *Clostridium perfringens* and *S. aureus* in the U.S., with the most common errors reported being inadequate cleaning of processing equipment or utensils followed by storage in a contaminated environment (39%). However, this review did not provide information on whether persistent strains were a significant issue.

Following four different staphylococcal outbreaks associated with desserts from an Illinois bakery, an investigation including several visits and sampling of environmental and raw ingredient samples was carried out. A raw ingredient and 16% of the environmental samples revealed the presence of enterotoxigenic *S. aureus* capable of producing diverse combinations of toxins. PFGE characterization of the isolates identified six pattern types (Hait et al. 2012). Similar findings, indicating persistence of numerous types of *S. aureus* in processing environments were made during another investigation related to two outbreaks (Hait et al. 2014).

Vázquez-Sánchez et al. (2013) investigated the influence of environmental stress factors found during seafood production on the adhesion and biofilm-forming properties of *S. aureus*. They concluded that the prevalence of *S. aureus* strains on food-processing surfaces was influenced by the ability to adapt to the environmental conditions present during processing, packaging, and storage of seafood products. Beneke et al. (2011) studied the fate of methicillin-resistant *S. aureus* in the fresh pork production chain, from the live animals through the slaughter and cutting lines to the final product. Methicillin-resistant *S. aureus* were identified at all stages of the production chain and could thus account for the presence in finished products. However, establishment in the environment was not necessarily indicated.

Outbreaks of *E. coli* O157 are often traced to animals, particularly cattle. The pathogen has been isolated from meat products, raw milk or agricultural products such as leafy greens, sprouts, apples used to manufacture juice, or nuts contaminated through exposure to fecal material, e.g., manure. Contaminated drinking or recreational water, direct contact with animals in petting farms, meats or direct person-to-person contact are sources of contamination (Pennington 2014) and a similar situation was observed for non-O157 Shiga toxin-producing *E. coli* (STEC) (Luna-Gierke et al. 2014). Data suggest that direct contact, rather than persistence in the environment is the primary concern.

While the persistence of STEC has been documented for farms and agricultural environments, little is known about its persistence in processing environments and processing lines. However, this route of contamination seems to play only a minor role. Marouani-Gadri et al. (2009) reported that the occurrence of resident microbiota isolated from meat site surfaces following cleaning and disinfection had a favorable effect on *E. coli* O157:H7 colonization of surfaces. However, a subsequent study (Marouani-Gadri et al. 2010) showed that persistence was not likely to occur when good refrigeration and hygiene practices were applied. Williams et al. (2008) determined that desiccation of surfaces in butcher shops reduced persistence of *E. coli* O157, e.g., dry sawdust spread onto floor tiles reduced, and damp sawdust increased survival and hence the risk of contamination of meat. Habimana et al. (2010) demonstrated enhanced colonization of food contact surfaces with *E. coli* O157 in the presence of *Acinetobacter calcoaceticus* which might contribute to additional contamination of raw meat as discussed by Giaouris et al. (2014) in a publication on the attachment and biofilm formation in meat processing environments.

A 2007 mixed-serotype outbreak of verocytotoxin-producing *Escherichia coli* (VTEC) O145:H28 and O26:H11 identified ice cream as the likely vehicle. It was produced at a dairy farm using pasteurized milk; and both serotypes were isolated from patients, ice cream, and environmental samples, suggesting post process contamination (Buvens et al. 2011). Murphy et al. (2007) and Čižek et al. (2008) investigated the contamination of milking machines and filters, focusing on up-stream segments of the line that are likely to harbor pathogens originating from the raw milk. Cagri-Mahmetoglu et al. (2011) investigated two cheese processing environments and found that *E. coli* O157:H7 was isolated less frequently than *L. monocytogenes* (2.7% versus 26% of the samples, respectively), a further indication that environmental contamination may also play a minor role for dairy-based products.

After an outbreak of *E. coli* O157:H7 associated with the consumption of ready-to-bake commercial prepackaged cookie dough, the extensive investigation of the processing environment and lines of the production facility as well as of the flour mill carried out did not identify a source, vehicle or production process that could have contributed to the contamination of the product (Neil et al. 2012).

An outbreak of *E. coli* O157 in Japan was linked to two varieties of rice cakes produced by a local manufacturer (Nabae et al. 2013). On-site and epidemiological investigations suggested that the contamination occurred during processing, but environmental samples taken from the manufacturing premises were negative for STEC.

Bacterial spore-formers are common contaminants of soil, water and raw materials used to manufacture products. The environmental conditions during which sporulation takes place have a strong impact on the heat-resistance of spores. Carlin (2011) reviewed the origin and routes of contamination of spore-formers, including a discussion on the role of processing facilities as sources. Huck et al. (2007) characterized the diversity and phylogenetic relationships among *Bacillus* and related spore-formers associated with raw milk processing lines up to pasteurized products in two plants. The same allelic types were frequently identified in paired raw milk and packaged product samples, indicating that *Bacillus* and *Paenibacillus* spp. can enter dairy processing systems through raw milk. Certain subtypes were found exclusively in pasteurized samples, suggesting the possibility of in-plant sources for these organisms, including through the persistence of selected subtypes in processing plants.

Alicyclobacillus spp. are a major concern as a spoilage microorganism in high-acid, fruit juice, fruit concentrate and blended juice products. The thermo-acidophilic nature of alicyclobacilli and the highly resistant endospores allow for their survival during the production of these products, leading to spoilage characterized by chemical off-flavors. Steyn et al. (2011a, b) reviewed alicyclobacilli in the fruit processing environment and the impact of its build-up as a function of the processing time.

Spore-formers in processing lines can contribute to cross-contamination of products during their manufacture. This has been reviewed by Evancho et al. (2010) for canned foods. Scott et al. (2007) and Burgess et al. (2014) studied the establishment, sporulation and build-up of thermophilic *Geobacillus stearothermophilus* spores in different sections of milk powder processing lines, such as the pre-heater or evaporator, thus leading to contamination of the final product. Studies on the occurrence of *B. cereus* in milk storage tanks (Shaheen et al. 2010), in pasteurized milk (Salustiano et al. 2009) and in cheese processing lines (Moradi-Khatoonabadi et al. 2014) illustrate the build-up and persistence of this organism in dairy processing environments and lines.

Post-process contamination of canned tuna fish and salmon with *C. botulinum* type E from within the canning factory has occurred (Johnston et al. 1963; Dack 1964; Denny 1982; Anonymous 1984). Contamination of low-acid canned foods with *C. botulinum* through contaminated cooling water has been investigated by Sachdeva et al. (2010) and Basavanna et al. (2013). While the introduction of *C. botulinum* in the cooling system through organic food debris or soil is possible, in this cannery, a rupture in an underground water line connecting the supply from two of the deep wells allowed the contamination of the cannery's cooling water supply.

12.4.1.3 No History of Establishment in Processing Facilities – *Y. Enterocolitica, S. Typhi, Shigella* spp., *C. jejuni,* viruses, parasites

Pathogens primarily of human origin (e.g., *S.* Typhi, *Shigella* spp., norovirus) have not been reported to become established in a modern manufacturing facility. Pathogens requiring a human host or living cells (e.g., viruses, parasites) cannot multiply in a food processing environment. Norovirus has been documented to persist for long periods of time on environmental surfaces in non-food processing environments (Bellou et al. 2012; Lopman et al. 2012).

Rimhanen-Finne et al. (2009) investigated a large *Yersinia pseudotuberculosis* outbreak associated with carrots. Although identical serotypes and genotypes of the outbreak strain were detected in environmental samples collected from the carrot distributor's storage facility, the authors concluded that the source of contamination remained unclear and an initial contamination in the field could not be excluded. A review of *Y. enterocolitica* outbreaks concluded that contamination from the processing environment has not been identified as source of contamination (Sabina et al. 2011).

Although *Campylobacter* spp. cross-contamination of prepared food in domestic and catering kitchens is a well-known phenomenon, establishment in food processing environments does not seem to occur (Silva et al., 2011). The high temperature and microaerophilic conditions required for growth and sensitivity to dehydration may preclude *C. jejuni* from becoming established in the processing environment.

12.5 Measures to Control Pathogens in the Food-Processing Environment

General approaches to control pathogens in the food processing environment include:

- Minimizing entry of pathogens into the processing environment,
- Minimizing establishment of pathogens in the processing environment, and
- Sampling the processing environment to verify control.

These topics are discussed below.

12.5.1 Minimizing Entry of Pathogens

It is not possible to totally prevent the introduction of pathogens into food processing facilities. This realization implies that GHP procedures should be targeted to control those pathogens most likely to be of concern for the food and conditions of operation. Among the sources of pathogens in food processing areas are:

- *Raw materials and ingredients* represent potential sources of pathogen entry into the food-processing environment. Raw agricultural commodities such as raw cocoa beans, milk, meat, poultry, fish, seafood, vegetables, fruit, nuts and spices represent important sources of pathogens to food processing facilities. Physical separation of raw materials through plant design and layout is necessary to minimize entry of pathogens into processed product areas.
- *Food handlers and maintenance personnel* can be a source of food contamination. Studies on the effectiveness of gloves and other measures to minimize recontamination suggest that training of personnel in hygiene is more important than the obligation to wear gloves. Despite this finding, both hand washing and disposable gloves are common in many manufacturing facilities. The impact of poor practices has been reviewed by Todd et al. (2007b, 2009).
- *Personal clothing* and, in particular, shoes can transfer pathogens from one area to another. While preventive measures can be adopted, such as changing shoes or boots, it may be more effective to design the plant layout to direct the flow of personnel. The use of footbaths to control pathogens on shoes, boots and equipment remains highly controversial. Some have found that maintaining clean, dry floors is more effective. Maintenance, management and control authority personnel whose responsibilities require them to routinely visit and inspect all areas of the plant can jeopardize control if they do not comply with established control practices.
- *Air and water*. Control of air quality is important in situations such as aseptic packaging, air chilling or drying, and air conveying systems. The filters for compressed air can become a source of contamination if not properly maintained. Aerosols created during cleaning can disperse microorganisms throughout the processing environment. General recommendations on air handling in food processing plants have been published by EHEDG (2005). Den Aantrekker et al. (2003a) summarized information on air contamination in different processing facilities and performed simulations to estimate the probability of aerial contamination, which could be useful to determine relevant control measures.

- *Water* as it enters a manufacturing facility should be potable but can become contaminated through use and serve as a vehicle for transmitting pathogens (e.g., water for washing, conveying, chilling) if not controlled.
- *Insects and other pests* such as flies, cockroaches or rodents can serve as vectors for pathogens and their role has been discussed by Greig et al. (2015). Numerous studies have been published on the association between specific pests and foodborne pathogens, including Wales et al. (2010), Baldacchino et al. (2013) and Barreiro et al. (2013). The role of insects as source of foodborne pathogens has been reviewed by Zurek and Gorham (2010) and Blazar et al. (2011). In food processing facilities, insects rarely cause direct contamination, but can act as vehicles for transmission.
- *Transport equipment* such as racks, carts, trolleys, forklifts or similar equipment, can be important vectors for transferring microorganisms throughout a facility. Some facilities color-code certain equipment and limit their use to specific areas.

12.5.2 Minimizing the Establishment of Pathogens in the Environment

Transport of pathogens into facilities is the first step in the process of pathogen contamination, but it is the establishment and multiplication of pathogens that increases the risk that food will become contaminated. Establishment in the factory environment may be linked to "macro" design and "micro" design, as well as adherence and colonization of microorganisms.

12.5.2.1 Macro and Micro Design

Food processing rooms (e.g., walls, ceilings, floors, windows, doors, conduits or trays for electrical cable, overhead pipes) should be designed, constructed and installed to minimize accumulation of dust and other material that can serve as sites potentially harboring microorganisms. They should be designed and installed to allow for efficient cleaning. Additional information is available, e.g., CAC (2003), EC (2004), and Lelieveld et al. (2014).

Surfaces of equipment, floors and walls may appear smooth to the naked human eye, but microscopic examination of materials such as stainless steel, rubber and plastic shows characteristic rough structures where microorganisms can hide and adhere. The microstructure of floors and walls is often rough or porous, and may deteriorate due to exposure to water, heat, chemicals and mechanical stress to form cracks and crevices.

Models have been developed that consider the hygiene aspects of food processing and to estimate the probability of recontamination (den Aantrekker et al. 2003b).

12.5.2.2 Adherence and Colonization: Biofilms and Niches

A range of factors contribute to microbial colonization of processing environments. When introduced into processing facilities, microorganisms may be on a carrier (e.g., dust, droplets, food particles) and, given the opportunity, attach to equipment or environmental surfaces. This initial attachment phase may be followed by adhesion. Adhesion depends on factors such as the type of surface (e.g., stainless steel, rubber), the type of microorganism and its physiological status, the physico-chemical status of the surface and the existing microflora. Exposure to acid conditions or mild heat seems to predispose cells and trigger the phenomena of attachment and adhesion. Individual cells adhering to solid

surfaces could be classified as biofilms (nascent biofilm). This status confers ecological advantages such as increased resistance against dehydration, heat and disinfection agents (Bridier et al. 2011). The most extreme forms of biofilms are found in systems with constant exposure to water, such as in pipes, flumes, air-cooling units, drains and floors. The more developed and complex (mature) the biofilm, the stronger the protective effect against environmental stresses, with protection being enhanced by the presence of biopolymers and other material.

Biofilms The formation of biofilms can occur in almost any environment with sufficient moisture and nutrients. Biofilms have been studied extensively on surfaces in contact with a liquid phase such as pipes, cooling towers and plate heat exchangers. Studies and reviews related to food processing environments include Van Houdt and Michiels (2010), Cappitelli et al. (2014), Bridier et al. (2014), and Kretli-Winkelströter et al. (2014). Research has been published for specific pathogens such as *L. monocytogenes* (Carpentier and Cerf 2011; Valderrama and Cutter 2013), *Salmonella* (Steenackers et al. 2012), *E. coli* O157 (Habimana et al. 2010; Vogeleer et al. 2014) and spore-formers (Abee et al. 2011; Faille et al. 2014). Studies on specific categories and processing environments are also available, such as for dairy processing (Bremer et al. 2009; Marchand et al. 2012), fresh-cut vegetables (Liu et al. 2013), produce (Jahid and Ha 2012), fish and meat (Jami et al. 2014; Sofos and Geomaras 2010).

Niches A niche is a site harboring microorganisms that typically is impossible to clean with normal cleaning and sanitizing procedures. The processing environment may appear visually clean and acceptable. Examples include hollow rollers on conveyors, cracked tubular support rods on equipment, the space between close fitting metal-to-metal or metal-to-plastic parts, worn or cracked rubber seals around doors, saturated insulation, interfaces between floors and equipment, cracks and crevices. Niches are frequently continually wet and hold residues of food and other material. Microorganisms, including pathogens, can become established and multiply within these sites, which then serve as a reservoir from which the pathogen is dispersed during operation, thereby contaminating food contact surfaces and food. In a controlled environment, the niche usually affects only the food along one processing or packaging line and not the product on close, adjacent lines. Microbiological testing is necessary to detect a niche.

12.6 Sampling the Processing Environment

Environmental sampling is used to:

- Assess the risk of product contamination,
- Establish a baseline for when the facility is considered under control,
- Assess whether the environment is under control, and
- Investigate a source of contamination so corrective actions can be implemented.

12.6.1 Assessing the Risk of Product Contamination (Investigational Phase)

Biased, investigational sampling techniques (see Chap. 11) are most appropriate for assessing whether a product has become contaminated with a pathogen. The purpose of this sampling is to find a target pathogen, if it is present. The selection of samples will reflect the experience of the investigator and the process being investigated. In general, in-process food samples and/or sponge samples may be collected. Food samples should be collected from stages that could permit contamination throughout the process. Consideration should be given to collecting samples at different production times (e.g.,

first product, middle of production, end of production, following a break in production, after mechanical repairs, after a change in ingredients or packaging material). Product residues (e.g., shavings from slicing machines, sweepings from the floor of a dry food facility, vacuum system material, and fines) can also be collected, since they serve as a type of composite sample from the process.

Environmental samples can be collected from the general processing environment as well as from product contact surfaces. Different techniques and tools are used to recover microorganisms from surfaces encountered in food processing environments and processing equipment. The choice of the most appropriate sampling tools and techniques should be adapted to the type and size of the surface to be sampled. Certain samples represent historical information, measuring accumulation over time. Examples include residues from hollow bodies, cracks and crevices; joints and seals between floor and equipment; water residues from siphons; drains; dust from vacuum cleaners; brooms, brushes and mops; air filters; and wet or old insulation material.

Sampling techniques applied in the food industry encompass a large number of tools. Non-antimicrobial sponges or large cotton pads are suitable for this purpose. Since the intent is to detect the presence of a pathogen, large areas should be sampled without regard to dimension. Environmental samples should also be collected at different times. Ismaïl et al. (2013) discussed advantages and drawbacks of frequently used methods. Techniques such as electrostatic wipes or roller samplers have been described, and results highlight the need for careful selection of sampling methods adapted to the conditions in the environment; e.g., dry or wet (Lutz et al. 2013). For example, Rönnquist et al. (2013) investigated the effectiveness of different types of swabs to recover norovirus from surfaces in food processing environments, and microfiber cloths appeared to give the highest recoveries.

In addition to analyzing for pathogens, samples may be tested for indicator microorganisms (e.g., *E. coli*, *Listeria* spp.) relevant to the pathogen(s) of concern. All the data should be organized in a manner such that a baseline can be established for what is considered normal when all GHP procedures are in control.

12.6.2 Establishing a Routine Environmental Sampling Program

Routine environmental sampling programs are usually focused on one pathogen or indicator microorganism and involve a limited sampling regime. The purpose of the routine sampling program is to detect increased risk of product contamination before it actually occurs. Data from the investigational phase are used to select sampling sites, times, frequencies, and types of samples that will most effectively fulfill that purpose. Some of the basic process control concepts described in Chap. 13 may be applicable in the design and implementation of an environmental sampling program. The use of trend analysis, in particular, is useful. Statistical analysis is seldom of value if the program involves presence/absence testing for a pathogen, such as in a cooked product area. In this case, any positive sample is a warning that requires further investigation.

12.6.3 Sampling Locations: The Zone Concept

Some manufacturers design their environmental sampling programs around zones having different levels of risk for product contamination (Fig. 12.1). For example, the zone that presents the highest risk of product contamination (Zone 1) includes product contact surfaces over or through which product passes during processing. The next zone (Zone 2) consists of equipment and other items that are in close proximity to the product flow and may indirectly lead to product contamination. Zone 3 may

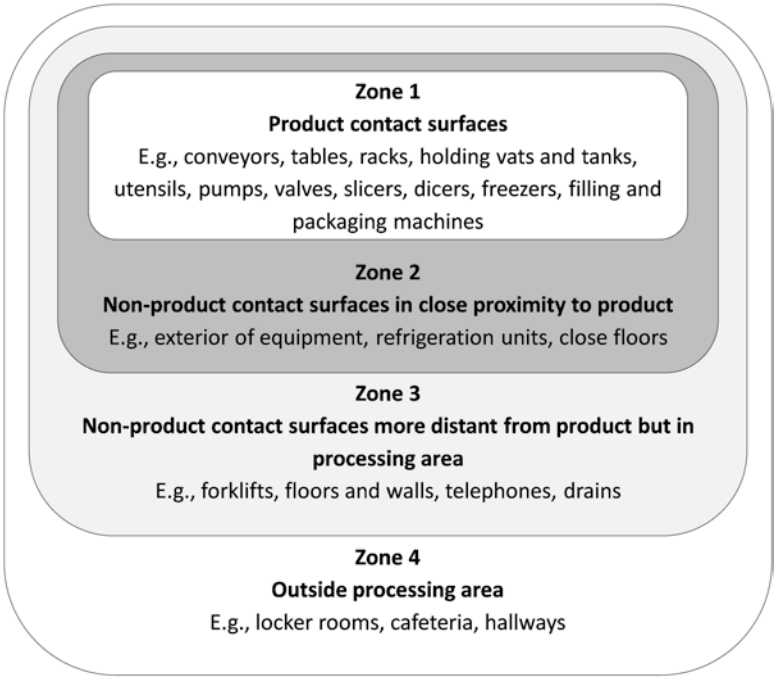
Zone 1
Product contact surfaces
E.g., conveyors, tables, racks, holding vats and tanks, utensils, pumps, valves, slicers, dicers, freezers, filling and packaging machines
Zone 2
Non-product contact surfaces in close proximity to product
E.g., exterior of equipment, refrigeration units, close floors
Zone 3
Non-product contact surfaces more distant from product but in processing area
E.g., forklifts, floors and walls, telephones, drains
Zone 4
Outside processing area
E.g., locker rooms, cafeteria, hallways

Fig. 12.1 Zone concept to illustrate areas of highest risk (Zone 1) to lowest risk (Zone 4) for product contamination

include items or areas that are less likely to lead to product contamination because they are more distant from the exposed product area; however, contamination in these areas may hinder efforts to control pathogens and potentially may transfer contamination to Zones 1 or 2 sites. Zone 4 is outside the processing area and if not maintained at an acceptable level of cleanliness could increase the risk of introducing pathogens into Zones 1–3. The concept of zones with differing levels of risk can be used to select sites for a routine environmental sampling program. In addition, the concept can be used as a teaching aid for plant personnel. It is important to recognize that the zone concept has not been standardized and the items designated for each zone differs among food operations depending on accumulated data and experience, as well as the type of facility (e.g., wet processing versus dry processing).

The purpose of routine environmental sampling is to verify that the GHP procedures are controlling the risk of product contamination, with sampling locations selected according to the risk of product contamination, as well as the likelihood of providing information to direct corrective action. In the case of heat-processed foods, for example, attention should be on the zones after cooking and where the products are exposed (e.g., where cooked ham is sliced and packaged). Emphasis should be placed on sites in Zone 1 and Zone 2, as needed. While contamination of a Zone 1 site presents a greater risk for product contamination, some environments have demonstrated a greater chance of detecting the target organism in Zone 2, which facilitates earlier application of corrective action before product is contaminated. Figure 12.2 illustrates possible sampling sites in four different food operations. Equipment located between when the food is smoked/heated and protected by wrapping or packaging is of greatest concern (designated by white boxes). Samples from product contact surfaces of equipment (i.e., Zone 1) that could be included in a routine sampling program are identified. In addition, samples from floors and other sites in close proximity to the flow of the product (i.e., Zone 2) are indicated (dark shading).

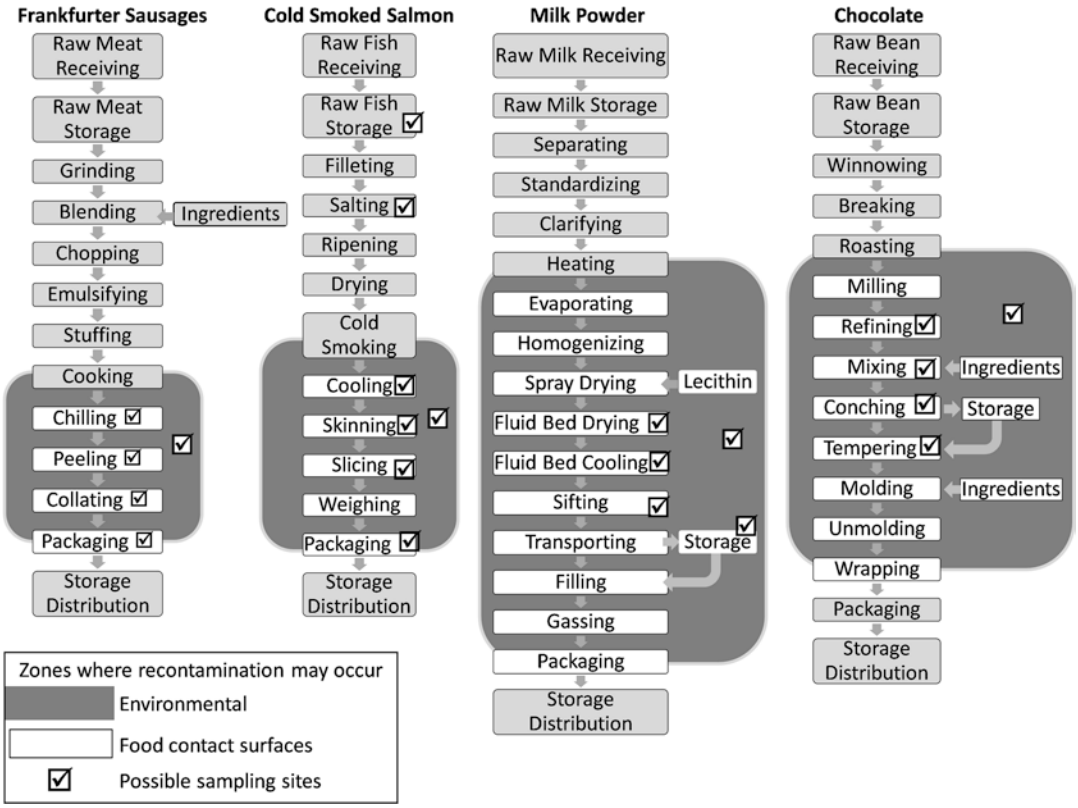

Fig. 12.2 Flow diagrams for four production lines and possible areas or sites to sample in the environment and on product contact surfaces of greatest concern for recontamination

The types of samples collected for a routine sampling program should be determined from the investigational sampling data and experience as the program is implemented. Environmental sponge samples are more commonly collected for analysis than product samples. Examples of sampling sites for four food operations are provided in Fig. 12.2. Environmental samples are intended to detect a microbial indicator or pathogen, if present. This may lead the sampler to collect from a large area from one piece of equipment and from a small area on another, relying on experience and previous results for guidance.

The sampling program could include in-process product samples where this may provide additional benefit over sponge sampling. The type of material sampled depends on the type of product and processing line. ICMSF (2011) provides recommendations on potential in-process and environmental sampling that may be useful for different types of products.

12.6.4 Number, Frequency and Time of Sampling

Environmental sampling protocols are not statistically designed sampling plans. Instead, they are based on experience and knowledge of the sites most likely to detect a failure in GHP. The knowledge base continues to increase over time, enabling adjustments to further improve their sensitivity without increasing analytical costs unnecessarily.

The number of samples and frequency of sampling are normally determined by knowledge of the operation and its variability. Knowing when to collect samples may be more important than increasing the number or frequency of samplings. For example, it may be known that sponge samples collected during operation will yield more useful information about whether the environment is under control than samples collected from cleaned equipment before start of operation, which may provide misleading results. In another situation, the first product from the process may represent the highest potential for detection of the target organism because it may remove residue that collected in interior surfaces that are not readily accessible for swabbing. Other important factors to consider include ease of sampling, whether the integrity of the product being processed will be jeopardized and, in particular, safety for the person collecting the samples.

Environmental sampling plans normally involve an established, routine sampling plan with a defined minimum number of samples. The number, frequency, timing and sampling sites may be increased when evidence indicates increased risk of contamination. Tables 12.3a and 12.3b list examples of sampling sites, numbers and frequencies for the operations for the four food products described in Table 12.2 and Fig. 12.2. In addition, the examples indicate a possible increase in frequency when

Table 12.2 Examples of possible sampling sites in the environment for four processing systems

	Frankfurters	Cold smoked salmon	Milk powder	Chocolate
Food contact surfaces (sponge samples)				
Equipment	Brine Chiller	Transport racks	After dryer	Mills
	Peeler table	Trays	After cooler	Refiner
	Casing removal system	Tables	Pipes	Conches
	Hopper after peeler	Skinner	Conveyor belts	Storage tanks
	Incline conveyor	Brine injector and tank	Tote bins	Pipes
	Collator	Slicer	Silos	Mixers
	Containers/tubs/trays	Scale		Molds
	Final Conveyor	Conveyor belts		Tempering equipment
	Packaging machine	Packaging machine		Transport trolleys
	Scale			Trays
Utensils	Knives	Knives	Brushes	Scrapers
	Tools	Tools	Scrapers	Paddles
		Scrapers		Spatulas
Environmental sponge samples (Indirect contact but close to product)				
Equipment	Overhead piping	Overhead piping	Lids	Lids
	On/off switches/buttons	On/off switches/buttons	Covers	Covers
	Legs of equipment	Legs of equipment	External surfaces of silos	Piping above equipment
	Sides of equipment	Sides of equipment		Internal surface cooling tunnel
	Bottom of equipment	Bottom of equipment		
	Floor by packaging line	Floor near packaging line		
	Floor of holding cooler	Floor of chill room		
Environmental sponge samples (Indirect contact)				
Environment	Floors	Floors	Floors	Floors
	Walls	Walls	Walls	Walls
	Refrigeration units	Refrigeration units	Pipes	Pipes
	Drains	Drains	Ducts	Ducts
	Doors	Doors	Overhead structures	Overhead structures
				Drains

data indicate such a need. When judged necessary, special samples can be collected such as during construction, during major changes to equipment, or after unforeseen events such as heavy storms that could have caused damage or contamination of the processing environment.

To minimize the analytical workload and cost, samples may be composited (i.e., bulked) before analysis (e.g., from the same sampling point over 1 week or from different sampling points along a processing line). Compositing should only be done when the quality of the information obtained is known to be unaffected.

Many facilities are adding or strengthening their pathogen environmental monitoring programs to enhance their food safety risk reduction efforts. The two most common types of pathogen environmental monitoring programs are *Listeria* spp. monitoring as an indicator for *L. monocytogenes* and *Salmonella* monitoring. Monitoring programs for other pathogens, such as *C. sakazakii* in infant formula manufacturing facilities, along with hygiene indicators such as *Enterobacteriaceae* (Buchanan and Oni 2012), share many similarities with the pathogen environmental monitoring programs discussed here. Monitoring for more generic indicator groups, such as sampling for total aerobic bacteria to verify sanitation, differs from the pathogen environmental monitoring program discussed here.

For food manufacturing facilities where there is a science-based reason for a pathogen environmental monitoring program, common components that should be built into the program to make it as effective as possible. Guidelines on pathogen environmental programs have been published by different organizations, e.g., GMA (2009) for monitoring *Salmonella* in low moisture foods, GMA (2010) for *Salmonella* in nut products, ABC (updated) for almond products, Tompkin et al. (2010) for *L. monocytogenes* in meat processing plants.

12.6.5 Sample Collection

Meaningful information is gained only if appropriate tools are used to collect samples. Sampling materials (e.g., sponges or cotton pads, utensils, cups, bags) must be sterile to avoid contamination. Proper labeling and description of the samples is essential. Before analysis, samples should be stored under appropriate conditions (see Chap. 9) so that the population of the target organism(s) neither increases nor decreases.

The sampling tools need to be adapted to the type of sample to allow for efficient sampling. Spatulas and scrapers of different size are used to collect residues and from surfaces, holes, crevices, etc. Paintbrushes can be used to collect dusty samples on surfaces of equipment or of the infrastructure (e.g. electric cables, control panels, etc.). Cotton plugs, sponges or gauze pads are useful for liquid or moist residues as well as surfaces of with limited amounts of residues. Sponges must not contain antimicrobial substances. Other sampling tools such as pipettes for liquids or spatulas for vacuum collection material may be required depending on the situation. Flexibility is needed to adapt the means of taking samples to local situations. The sampling tools must not introduce other, non-microbiological, hazards into the processing system. In most manufacturing plants, for example, a strict policy exists that glass cannot be introduced into the processing environment. ABC (undated) provides illustrations on the types of tools that could be used in different locations in almond production.

12.6.6 Sample Analysis

Samples taken for microbiological surveillance are analyzed for specific microorganisms; i.e., pathogens of concern (e.g., *Salmonella*, *L. monocytogenes*) or indicators such as non-pathogenic *E. coli.* Usually, traditional microbiological methods are used, although some more rapid methods are gaining acceptance.

A routine testing program for a pathogen may be supplemented with tests for hygiene indicators. Pathogen testing is usually qualitative (presence/absence), which can be time consuming, costly, and cumbersome. Therefore, the potential presence of pathogens may be assessed using indicators. Quantitative determination of *Enterobacteriaceae*, for example, allows assessment of moisture control in environments intended to remain dry. The use of biochemical reactions to detect ATP (Carrascosa et al. 2012; Sharma and Malik 2013) or residual proteins enlarges the concept of hygiene indicators. Although not directed towards the detection of viable microorganisms, the presence of residues of the manufactured products on wet cleaned equipment indicates the potential presence of nutrients to support microbial growth, which may contribute to recontamination.

Of particular interest for environmental monitoring are sub-typing methods, which are used for pathogens and increasingly for other microorganisms. While biochemical methods are of limited use, serological, biological (phage-typing) and molecular methods such as PFGE, RAPD (randomly amplified polymorphic DNA), ribotyping and others are finding more applications. Whole genome sequencing, which is now being routinely used for molecular typing by agencies such as the CDC, U.S FDA, U.S. FSIS, and the Public Health Agency of Canada, will eventually replace the molecular typing methods now in use. These methods, especially the molecular ones, are very powerful tools to trace microorganisms within processing facilities and to establish links between isolates. Reviews of available methods and techniques have been published (e.g., Sabat et al. 2013; Oyarzabal and Kathariou 2014).

12.6.7 Managing the Data from Environmental Sampling

An environmental sampling program is useful only when data acquired are organized and frequently reviewed. In addition to reviewing the most recent data, it is also helpful to review data for the past quarter or year to detect weaknesses and trends that may not be otherwise be evident. Normal, routine levels of sampling can be continued as long as the data are within the limits of acceptance. Detection of increased levels of indicators such as *Enterobacteriaceae* may not trigger immediate corrective actions, but should serve as a warning. Ongoing computational analysis of environmental testing results lends itself to software assisted warning systems. The response depends on the type of product manufactured.

12.6.8 Investigational Sampling to Determine the Source of Contamination

When a trend or other information indicates an increased risk of contamination, the reason should be determined. This involves a combination of increased sampling and biased, investigational sampling leading to collection of the data necessary to identify the source of contamination and to implement corrective actions. Tables 12.3a and 12.3b provide examples of increased levels of sampling that could be used for the four processes introduced in Fig. 12.2.

During investigational sampling to detect the source of contamination, it is important to assemble and review historical environmental sampling data that may reveal trends or suggest a particular cause. Of particular interest are data for finished product, ingredients and the processing environment. These data may not include tests for the microorganism in question, but correlating data may provide insight into changes in the microbiology of the ingredients, process or the environment. In addition, production records may associate the problem with specific events such as construction, remodeling, mechanical repairs, and tests of new equipment or product formulations. In addition, specialists with appropriate experience can conduct visual inspections of the cleaning and disinfecting procedures, the

Table 12.3a Example of an environmental sampling plan for *L. monocytogenes* in a frankfurter and a cold smoked salmon operation

			Normal level	Increased level
Frankfurter plant	Environmental sponge samples	Floor in peeler area	1× week	1–3× day
		Floor in vicinity of collating and packaging line	1× week	1–3× day
	Equipment sponge samples, etc.	Brine chill solution	1× week	1–3× day
		Peeling table	1× week	1–3× day
		Hopper/incline conveyor after peeler	1× week	1–3× day
		Collator	1× week	1–3× day
		Conveyor before packaging	1× week	1–3× day
		Packaging machine	1× week	1–3× day
	Finished product		1× bi-weekly	1–3× day
Cold smoked salmon plant	Environmental sponge samples	Floor of chill room	1× week	1–3× day
		Floor in vicinity of slicing line	1× week	1–3× day
	Equipment sponge samples	Brine injector/brine tank	1× week	1–3× day
		Racks exiting chiller	1× week	1–3× day
		Conveyor/table	1× week	1–3× day
		Skinner	1× week	1–3× day
		Slicer	1× week	1–3× day
		Scale	1× week	1–3× day
		Packaging machine	1× week	1–3× day
	Finished product		1× bi-weekly	1–3× day

Table 12.3b Example of an environmental sampling plan for *Salmonella* in a dried milk power and a chocolate plant

			Normal level	*Increased level*
Milk powder plant	Environmental sponge samples	Area zone drying tower	1× week	Several points
		Area zone after dryer	1× week	Several points
		Area zone storage silos	1× week	Several points
		Area zone packaging	1× week	Several points
		Star valve	1× week	1× day
		Filters	1× month	Several points
		Cyclone residues	1× week	1× day
		Sieves after cooler	1× day	Several points
		Silos	1× week	1× day
		Packaging machine	First product	10× day
	Finished product		3× batch	10× batch
Chocolate plant	Environmental sponge samples	Area zone after roaster	1× week	Several points
		Area zone refining	1× week	Several points
		Area zone mixer	1× week	Several points
		Area storage rework	1× week	Several points
		Area storage ingredients	1× week	Several points
	Equipment sponge samples	Mixer	1× week	1× day
		Conches	1× day (rotation)	1× day (all)
		Tempering equipment	1× day (rotation)	1× day (all)
		Storage tanks	1× day (rotation)	1× day (all)
		Molding	1× week	Several points
		Rework vessel	1× week	1× day
	Finished product		3× batch	10× batch

status of the equipment and operating conditions, employee practices and movement of equipment, product and personnel.

The goal of the intensified sampling is to detect the contamination and its cause. This type of sampling may identify e.g., biofilm formation, or a niche supporting microbial growth in the equipment. When pursuing a source such as a niche, it is usually necessary to completely dismantle suspected equipment. As this is being done, samples should be collected to confirm, if possible, that the source has been found. In such situations, care must be taken to avoid dissemination of the pathogens dislodged from their niche into the environment by careless handling of material and samples.

The increased frequency of sampling is normally not restricted to samples of the same type taken at the usual location. In reality, the source of the pathogen needs to be investigated to determine whether it is a transient or resident strain in the factory environment. Tracing, source detection, or troubleshooting can be a long and tedious process requiring repeated sampling campaigns, where attention is focused on sites in addition to those included in routine sampling. Pre-conceived ideas about the source should be viewed with caution and have been known to prolong the effort of detecting the true source. Sampling may be expanded to adjacent zones of lower concern to obtain a more complete picture of the extent of the contamination. Dismantling equipment, removing filters, etc. may be necessary to access sites that could harbor a niche.

Two unpublished case studies of investigational sampling in problem solving are provided below.

Listeria monocytogenes in processed, cold-filled cheese In this example, *L. monocytogenes* was isolated from processed cold-filled cheese. Upon investigating the source, 80 finished product samples from a one-month period, over 200 ingredient samples and 1230 environmental samples were analyzed. *L. monocytogenes* was found in 6 finished product samples, 3 ingredient samples and 1 environmental sample, some with multiple isolates. The only ingredient found to be contaminated was butter from a particular supplier. The single environmental sample that was positive, was from the underside of an elevated electrical cable stand to a chopper. All 14 isolates were of the same serotype (1/2 b), multilocus electrophoresis type (ET202), ribotype (DD0941) and phagetype (non-typable). The probability that all 14 isolates would by chance have the same pattern is extremely remote and indicates a common source of contamination. Most likely, the butter was the source of contamination for the processed cheese and the single environmental positive resulted from spilled butter or cheese during manufacture. Since only one of the 1230 environmental sponge samples was positive, it can be concluded that the unique isolate was not a resident of the facility producing processed, cold-filled cheese. This study illustrates how serotyping, electrophoresis, ribotyping and phage typing can be used in investigations to detect the source of contamination.

Staphylococcus aureus *in fermented sausage* High numbers (e.g., $> 10^5$/g) of *S. aureus* detected during a routine verification at the end of fermentation for a dry fermented sausage could have been due to a variety of reasons. Product produced before and after the suspect lot was analyzed to determine if the problem was a continuing or an isolated problem. Furthermore, individual lots produced during the period in question were sub-lotted into smaller batches by time of production. Review of the processing records for the incriminated lots helped to determine the origin of the problem. In addition, equipment and environmental sampling was conducted. Since *S. aureus* numbers decline during the drying/aging step that follows fermentation, quantitative analysis for *S. aureus* might yield misleading information. Thus, the product was tested for heat stable thermonuclease as an indicator of high numbers of *S. aureus*. The samples were collected from the outer few millimeters of the sausages, since this is where growth of *S. aureus* would occur. The accumulated information indicated that the products most likely to contain detectable levels of heat stable thermonuclease were the first batches produced after weekend shut downs. Sponge samples from equipment initially failed to detect a source of contamination; however, the production records clearly suggested growth of *S. aureus* in the processing equipment during shutdown as the probable cause. Re-examination of the equipment revealed growth in an area of the equipment that was difficult to reach and clean (and sample).

The investigation concluded that *S. aureus* multiplication occurred in a niche in the equipment during shutdown on weekends. Meat moving through the unclean equipment during start of production on Monday mornings became contaminated with relatively high numbers of *S. aureus*. As production continued, subsequent batches were contaminated with decreasing numbers of *S. aureus*. The lower levels did not reach unacceptable levels during fermentation.

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Chapter 13
Statistical Process Control

13.1 Introduction

Food operations must be controlled to produce foods of consistent quality and safety. A controlled process requires process managers being proactive and informed of the factors that influence variability. Process control thinking and technology can be applied to the manufacture of a single lot of food produced on 1 day, or multiple lots produced over days or years, and to both batch and continuous processes. This chapter discusses sampling and testing to assess whether food process operations are under control (i.e., correct procedures are being followed and production/manufacturing conditions are being met) and using the data to make the adjustments necessary to maintain control through the use of statistical process control (SPC) methods. Results collected from using industrial process control technology systems can be analyzed through using SPC techniques to assist with process assessment.

As described in Chaps. 3, 4 and 5, the process or product criteria that are used to assess whether a food safety system is under control can be based on an FSO, PO or a performance criterion and will be specific to the food, the process, and the microbial hazard(s). Criteria can be standards, guidelines, specifications or whatever a regulatory authority, purchaser or processor considers necessary to ensure a process is controlled and the food will be safe when used as intended.

From the early 1990s, there has been increasing interest within the food industry in quality enhancement through programs that stress the use of structured quality and food safety management systems. The concept of continuous improvement has led to greater use of data in a more organized manner to improve quality and production efficiencies. This interest in quality systems occurred during a period when HACCP was being more widely adopted throughout the industry. HACCP systems are, in essence, that portion of an establishment's overall process control system that focuses on food safety. Collectively, these programs have led to a greater awareness of the need and value of process control and applying statistical methods to analyze and interpret data. While food safety will be emphasized below, the concepts described can be also applied to ensuring microbial quality.

Microbiological testing is conducted at various points along the food chain for a number of different purposes. Some of these are summarized in Tables 1.2 and 5.1. In Chaps. 4 and 5, microbiological testing for evaluation of lots or consignments of food (raw materials and end products) in commerce is discussed. However, due to the time required for most microbiological analyses and the relative insensitivity of even the most stringent sampling plans, microbiological testing is of limited value for monitoring in quality and safety assurance programs (NRC 1985; ICMSF 1988; NACMCF 1997)

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International Commission on Microbiological Specifications for Foods (ICMSF),
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(also see Chaps. 6, 7, and 9). More rapid tests must necessarily be used and will typically involve sensorial, chemical and/or physical measurements such as time, temperature, acidity, pH, moisture, a_w, flow rates etc. The principles of SPC described in this chapter can be applied to all of such measurements. Thus, while the emphasis of the book is on microbiological testing, it should be kept in mind that other measurements will more commonly be used to monitor variability in process systems (see Chap. 4).

HACCP is often referred to as a preventive system. However, from a statistical standpoint HACCP would be more appropriately described as a means for controlling the variability of a food system, as monitoring control points (CPs) and critical control points (CCPs) typically will result in reduced variance of a particular process parameter (ICMSF 1988). A statistical approach to safety can be effectively applied in HACCP systems. SPC methods provide an objective and statistically valid means to assess ongoing processes, and as such are particularly applicable to monitoring and verification of a food system.

Lot acceptance sampling can be used in verification and, to a very limited extent, in monitoring (e.g., sampling of shelf stable dry foods that can be held until the results of microbiological testing are available). SPC and lot acceptance sampling are two statistical techniques for controlling quality and safety are well developed and documented in many textbooks, manuals and periodicals (ASTM 1951; Duncan 1986; Grant and Leavenworth 1972; Massart et al. 1978; ICMSF 1986, 1988). The statistics of lot acceptance plans have been dealt with in detail in Chaps. 6 and 7. SPC methods with applications to food technology are treated in detail by Kramer and Twigg (1982), Hayes et al. (1997), Merton Hubbard (2003), Augustin and Minvielle (2008) and Lim et al. (2014).

This chapter is intended to serve as a brief introduction to the use of statistical methods for SPC. For information on process control technologies, other texts are recommended (ICMSF 2011). Further, for more information on process-oriented control systems in the food industry and the statistical tools used for that purpose, other texts are recommended (Steiner 1984; Hubbard 2003; DeVor et al. 1992; Juran and Gryna 1988; Montgomery 2009, Wheeler 2010). As we move forward with this discussion on SPC in the food industry, it is important to note that while SPC techniques can be used for many aspects in processing including process parameters as well as environmental parameters, the reader should consider the SPC discussions herein to be mainly focused on process parameters (Hayes et al. 1997; Augustin and Minvielle 2008).

Note
In our discussion in this chapter, the term "monitoring" is used to describe how a parameter is following the process from a statistical viewpoint and in most instances would be similar to "verification" used in process control in other parts of this book. This interchange of words is an artifact of the standard terminology used in SPC versus that used in control measures related to food safety. An effort will be made to clarify the particular use of these terms during further discussion in this chapter.

The application of SPC to food safety relies heavily on a detailed understanding of the hazards, the food and its ingredients, and the processes employed in its manufacture. There is no substitute for wide-ranging and complete knowledge of a process system. To the extent possible this process knowledge should be captured electronically. There are many techniques to assist process managers in this task: process flow diagrams and process mapping techniques (Montgomery 2009) are good resources to use to create one's process knowledge base. A well documented, described and understood process is the foundation of the elements listed below.

1. Knowledge of the significant hazards: The principles of HACCP should be considered for all food operations. At a minimum, a hazard analysis should be conducted. Data acquired as part of SPC monitoring of process parameters can lead to better process understanding, identification of significant hazards and parameters contributing to overall process variability.

2. *Knowledge of the factors that are necessary for control*

If significant microbial hazards have been identified, it is necessary to assess the conditions of processing and measure or step that must be controlled to prevent, eliminate or reduce their occurrence to acceptable levels. Subsequently, control measures can be established that adequately and consistently ensure the required level of control. This will help designate the importance of certain steps in the process i.e., as CCPs, CPs or other prerequisite programs including GHP procedures for controlling the identified hazards. The use of SPC methods can aid in identifying the factors and process parameters to control and eliminate hazards.

3. *Knowledge of the extent of variability and factors that influence variability*

Key to using SPC methods is understanding the factors that influence variability. Most food operators understand the factors that influence the cost of producing a food and strive to control each factor according to their relative impacts on cost and profit. Likewise, this concept can be applied to producing safe foods. Processes with a high degree of variability, particularly when that variability is not recognized or understood, are more likely to produce unacceptable, and possibly hazardous food. Each process is unique owing to differences in plant layout, equipment design and performance, equipment maintenance and cleanability, personnel, type of food being produced and other factors. The conditions that influence variability at CCPs must be understood as well as the degree of variability that can occur. The information could, through the use of SPC, then be used to determine how this variability might be controlled within an acceptable range. This should include both short term variability and variations that can occur over time such as seasonal changes in the levels of organisms associated with various ingredients.

4. *Establishing criteria for the factors that must be controlled*

The information from item 3 above should be used to establish operating parameters that take account of variability and ensure that critical limits are met. Through continuous improvement, variability can be reduced and result in improved safety, quality and process efficiency. With limits having been established at critical steps in the operation, procedures must be established to monitor those limits to ensure they are met during operation. By using SPC charting along with information on what to do when a system is out of control, discussed later as an "out of control action plan" (OCAP), factors to be controlled can be charted, followed and studied.

5. *Establishing monitoring procedures*

A wide variety of measurements such as sensorial, physical, chemical and environmental are used for evaluation of food processes. The chosen method will typically be the simplest, easiest, cheapest and safest available that can provide, in a timely manner, the information needed to adjust the process and maintain control. Ideally, the measurements would be continuous with adjustments being made automatically. The measurements may include processing parameters (e.g., weight, temperature, humidity, pH), food collected at different stages in processing, finished product, and/or environmental samples. A permanent record should be created for subsequent verification. SPC methods can be used to monitor data, establish these records, and provide trend analysis capability.

6. *Organizing and interpreting data*

Considerable quantities of data are generated to evaluate current production and when organized correctly, the data have even greater value. If properly organized, the data can be used to determine

longer-term trends and facilitate continuous process improvement. With the availability and power of computational and modern informatics systems (e.g., LIMS that allow the direct capture of data being generated by various sensors in a facility), data can be organized into electronic databases that allow rapid interrogation and provide cumulative and exhaustive records. Recent advances in "big data" management provide means for the analysis of many factors that previously could not be realistically considered.

7. *Using the data to measure change and improve control*

The value of an effective process control system is most evident when data are organized and used to further increase knowledge about a process and the factors affecting variability. The longer-term goal should be to use the data to reduce variability and achieve more tightly controlled processes. Properly organized, the data can be used to measure the effect of modifications to equipment and other factors in the process. In addition, the data can be used to detect trends over multiple lots of food that may indicate a gradual loss of control. When data are organized and routinely reviewed, adjustments can be made to maintain control during production of one lot or across multiple lots of food.

8. *Responding to the data*

Ideally after a process has been modified, data will indicate reduced variability and improved statistical control of the processing system. This re-affirms the operator's understanding of the factors affecting variability and how the process may be further improved. Occasionally, trends pointing towards deviation indicate the need to determine which factor has changed and requires correcting. Responding to these changes in the data is a key to better process analysis. Further, with computer assisted trend analysis, informatics system can be automated to provide warnings of changing conditions that warrant investigation.

9. *Investigating and learning from previously unrecognized factors or unforeseen events*

Occasionally, an unexpected change occurs in a process that results in loss of SPC. Upon investigation, the cause is determined and controls are implemented to prevent a similar event. This could occur through equipment malfunction and lead to a new preventive maintenance procedure to avoid future malfunctions. Another possibility could involve weather changes (e.g., higher humidity in summer compared with winter and its effect on various processes) or power outages from storms or shortages of electricity in a region. Isolated events may result in no change to an existing process control system as long as the process monitoring procedures and confidence in control are not affected, but more frequent occurrences may require a prepared plan of action.

Items 1 and 2, above are well known as the first two principles of HACCP (ICMSF 1988; CAC 1997; NACMCF 1997) and are not discussed here. The next section provides the reader with a general introduction to statistical process control (SPC). The sections that follow provide examples of how SPC methods can used for process monitoring and verify food safety operations.

13.2 Statistical Process Control: An Introduction

Statistical process control has been used in the manufacturing setting for many years for controlling product quality. A simplified presentation of the basic principles of SPC will be given to set the stage for techniques introduce later in the chapter. Interested readers are encouraged to consult additional texts for more depth consideration of the topic (Montgomery 2009; Wheeler 2010; Levinson 2011).

Seven basic tools provide the foundation for a SPC program (Montgomery 2009):

1. Basic process descriptions (i.e., know your process);
   - basic statistics and related distribution analyses
   - DMAIC process (Define, Measure, Analyze, Improve, Control)
   - hypothesis testing methods
2. Check sheets
3. Pareto charts
4. Cause and effect diagrams
5. Defect concentration diagrams
6. Scatter diagrams
7. Control charting

The presentation of these tools is to provide the reader with a basic understanding that a SPC program is more than just control charting of process data. Statistical process control is a realization that the process owner must "*know your process*" and all of these tools have an impact on overall process control. The emphasis in this chapter is how to effectively use an SPC program to monitor process data. These SPC tools are considered to be "*voices of the process*" in that they reflect the process as well as parameter means and variabilities.

A number of the tools of SPC programs already exist in many food operations today. For example, check sheets and scatter diagrams are used to identify potential areas for further process monitoring, process improvements and data relationships. The chapter primarily focuses on 'control charting' (tool 7, as above). The interested reader is encouraged to look at other previously mentioned texts for further information on the other SPC tools. Where appropriate, examples will be given of control chart types that one might use in industrial situations. This is not an exhaustive list.

13.3 Knowledge of the Degree of Variability and the Factors that Influence Variability

13.3.1 Establishing a Baseline for a Process

Food manufacturers collect a variety of samples for microbiological analysis including samples from equipment, the processing environment, ingredients, in-line food samples and finished product. The selection of samples and choice of analyses is influenced by the type of food and food operation. Microbiological analyses of samples should generate data that can help the process and/or quality manager assess the degree of control in the production facility and if needed, the corrective actions required to ensure safe and compliant food. Since one or more days may elapse between sampling and obtaining a result from a microbiological analysis, the data provides a history of past performance. While often not clearly articulated, the purpose of this sampling is to provide a "microbiological history" of the food product and the processing conditions (Buchanan 2000) as well as verification of the effectiveness of hygiene control measures. By acquiring data over time on the microbial population, manufacturers establish a baseline for the level of control that is attainable when GHP procedures and the HACCP system are in control. Once established, subsequent analyses that differ from the baseline indicate a deviation from the norm due to changes in operating conditions, including OPRPs. Additionally, these data can form the basis of manufacturing trends that might be useful for historical analysis and analysis of process deviations.

The extent of microbiological testing for verification is typically limited and not intended to provide assurance of the safety of any specific batch or lot of food. If sufficient data have been accumulated

over multiple lots, statistical analysis can greatly enhance the usefulness of the data. This type of analysis, sometimes referred to as "cross-batch testing," is similar to data for a single batch, except the data are collected over time and involve multiple batches. An underlying assumption is that when a process is statistically in control, the "between-batch" variability is small and the overall variability is stable.

13.3.2 Types of Microbiological Data for Baseline

Microbiological data can be collected from at least five sources that differ by location or time of sampling within the process (i.e., ingredients, in-line samples, end product, equipment/environmental samples, and shelf life samples).

Ingredient data can be viewed as a type of in-line sample. Periodic microbiological testing of ingredients can be used to verify that a step in the process that is not directly under the control of the manufacturer is, in fact, in control. The microbiological specifications for ingredients are typically defined through purchase specifications. This often requires the supplier to provide a "Certificate of Analysis" (COA) for ingredient batches. Additionally, periodic microbiological testing of ingredients by the purchaser is a means of verifying that the supplier is meeting the specifications. As an aside, lot by lot microbiological testing for release by the supplier would be considered the equivalent of a CCP, whereas the same testing done periodically by the purchaser would be considered testing for verification. Ingredient testing is particularly important when microbial levels could potentially be so large as to overwhelm the controls designed into the food safety system or when a sensitive ingredient is used in a process that has no kill step (e.g., blending dry ingredients for powdered infant formula and chocolate manufacture).

In-line sampling consists of collecting samples of the food at different steps in the process. The data provide information needed to understand the effects of each step on the microbial population. The location (e.g., at start, middle, or end of production) and time (e.g., beginning of the day, shift sampling, and others) at which in-line samples are collected may be important. Sampling before and after a critical step in a process can be used to verify the effectiveness and variability associated with control measures. Although end product testing is a common sampling location, by itself it rarely provides the needed information. Analysis of the finished product provides an integrated measure of all the steps that contributed to the total population, but if the product does not meet a specification the results do not identify the cause of the problem. In-line samples may be necessary to identify the cause and periodic in-line testing helps speed root cause analyses after a process deviation.

As previously indicated *end product* testing is generally not necessary on a routine basis (i.e., as a monitoring step or control measure) when records indicate a process is in control. However, periodic verification may be useful, particularly in combination with in-line sampling, as a means of trend analysis or root cause analysis in the case of process deviations. The merits and limitations of end product testing as a measure of process control is discussed in Chaps. 4 and 5.

Equipment and environmental tests are used to measure the effectiveness of GHP/GMP within a food operation. Visual inspection is the most common method of monitoring whether equipment has been adequately cleaned/sanitized between production runs, though this is increasingly being augmented by chemical detection systems such as the determination of residual ATP. Many operators routinely supplement the visual inspection with sponge or swab samples collected from the equipment as a means of verification. The sampling sites and frequency of sampling are determined by experience and whether problems are detected. In other operations, an inspector decides when and where to collect microbiological samples, usually only when there is uncertainty or it is desirable to confirm an observation.

The value of environmental sampling for microbial control is discussed in Chap. 12. The correlation between the microbiological status of the environment and a food is highly dependent on the

characteristics of the food manufacturing system. For example, an enclosed processing system has fewer opportunities to become contaminated from the surrounding environment; the relationship between environmental samples and the product being tenuous. Conversely, food production systems with a great deal of manipulation and exposure to the plant environment are more susceptible to environmental contamination and there will more likely be a stronger correlation between microbes detected in environmental samples and the microbiological quality or safety of a food.

Shelf life sampling is a specialized form of end product testing that involves holding finished product for longer than the time specified on the package and then examining it for specific attributes. Shelf life testing is most often used to establish code-dating practices based on quality attributes (e.g., time to spoilage). However, shelf life testing can be used to evaluate whether the levels of certain pathogens can increase, remain unchanged or decrease during the normal expected conditions of storage and handling. If the pathogen is initially present at levels where detection by standard microbiological analytical methods is unlikely, holding the product under "market or use conditions" can offer one means of estimating consumers' potential exposure at the time of consumption. Accelerated shelf-life testing (e.g., incubating the food at an elevated temperature) may be useful if the response after accelerated storage can be correlated to what occurs during the normal distribution chain, storage and use.

13.3.3 Determining the Causes of Variability

Variability can occur for many reasons. There may be inherent differences in an ingredient from one lot to the next. Solid foods may vary in dimension or weight. Liquids and semi-liquids may differ in viscosity and other attributes. The variety, species or age of an ingredient can introduce differences in composition, texture and other properties of the food that is being processed and therefore contribute to variability. Equipment maintenance and performance can be a significant influence on variability. Facilities having the same equipment will likely experience different degrees of variability depending on equipment age, model, maintenance, etc. In certain regions, seasonal and weather effects can significantly affect humidity and drying conditions such as for dry cured hams and fermented sausages and legumes and cereals during harvesting and subsequent storage. The composition of milk (e.g., fat content) varies with season and must be taken into account when manufacturing cheese. The factors having a significant impact on variability must be determined for each food operation and a decision taken whether those factors are sufficiently important to be controlled. The food safety system must take account of variability when establishing limits and monitoring procedures and be sufficiently conservative to ensure the food being produced will be acceptable and safe.

13.3.4 Variability in Foods and Food Processing Parameters

There are two distinct patterns of variability in food characteristics and in the control parameters of food processing systems. The first applies to intended characteristics or process parameters such as pH, temperature, or even the concentration of intentionally added microorganisms in fermented products. In this pattern, there is a *target value* and some degree of *variation* above and below that value. These intended characteristics/parameters are controlled by keeping the mean value for the process near the target value and focusing on minimizing the spread (variability) above and below the mean.

Intended characteristics or parameters of a food or a process are usually controlled in terms of the Normal distribution, which is derived from a mathematical theorem called the "Law of Large Numbers" (Sen and Singer 1993). That theorem states, in simple terms, that a collection of mean

values from a large number of samples, all from the same type of original distribution, will show the variation described by the Normal distribution, the typical bell-shaped curve. Since the Normal distribution applies to mean values, a great deal of SPC theory is based on the distributions of the mean values of multiple measurements. This analysis has proven both reliable and valuable.

The means of even a small number of samples of a distribution are often nearly normally distributed. For many intended food characteristics or control parameters such as pH and temperature, the distribution of individual values is essentially normal even when the number of samples being considered is relatively small, e.g., n = 5 (Montgomery 2009). In fact, for many characteristics or attributes in nature such as the pH of an individual sample or the population density of bacteria in a growth medium, an individual sample can be viewed as the outcome of many small samplings (i.e., means). Therefore, if one can represent the parameter or characteristic in the correct units, the distribution of individual results will approximate a Normal distribution. In the case of bacteria cultured in an environment that supports non-steady state growth, such as occurs in a food, the increases in population density typically occur exponentially. Declines under adverse conditions are also roughly exponential. It has long been observed that "concentrations" of microorganisms have a Lognormal distribution, and consequently have a Normal distribution when concentrations are expressed as logarithmic values.

The second pattern of variability applies to unintended and undesirable characteristics such as concentrations of spoilage microorganisms or specific pathogens. In this instance, the goal is to keep the level as low as possible based on technological, economic, and/or public health considerations. In the case of infectious pathogens, the target value is often the absence of the biological agent as analyzed by a specific microbiological assay in a given weight of product. However, even when the target value is absence in a specified quantity of food, there is some distribution of values inherent in the target. The rare chance occurrence of a pathogen is not necessarily indicative of the process being out of statistical control. Typically, target values for indicator organisms (e.g., coliforms) are as low as can be achieved with GHP and HACCP and, when present, are within levels considered acceptable for the process and food. Control of unintended characteristics such as microbiological contamination is usually achieved using two targets: a limit on the *fraction of product that shows contamination* and an upper limit on the *concentration of contaminants* that occurs in that fraction. One method to obtain quantitative measures of these limits is to perform a process capability study (see Sect. 13.4).

13.4 Process Capability Study

Confidence in the performance and variability of individual processes, or entire systems, is based on data from past performance, often through the development of control charts. One may compare these control charts to previously determined microbiological baselines. The use of historical, product development, and/or the initial control chart data to set warning and action control limits is termed a "process capability study". Such studies can be considered part of the process validation activities that establish the efficacy of a new process or process step to control a microbial hazard. Like the HACCP plan itself, a process capability study is only valid for the process on which it was conducted. A change in the process that might alter product safety would require a new process capability study, which would in turn validate the process. Ideally, validation, through a process capability study, would be of sufficient duration to quantify all factors (e.g., seasonality, alternate sources of raw materials) that could influence process performance. However, this is seldom achieved so subsequent verification activities are designed to acquire additional data to enhance the initial process capability study. A process capability study has two parts:

- collecting data that show the distribution of measurements when a process is operating as intended;
- using the data to derive one or more process limits on sequential patterns of data.

Whether for attribute (i.e., presence/absence or stratified numerical) data or microbial numbers (variables data), the setting of microbiological limits will ultimately be a matter of judgment, experience, and will consider the risk and consequences of failure. A process capability study of an attribute associated with a process parameter often could be useful for establishing the critical limit, e.g., temperature of pasteurization. Similarly, a process capability study that examines the frequency and extent of contamination by a specific pathogen or indicator microorganism for a process could provide the basis for establishing criteria for verification. Several countries have acquired "national microbiological baseline" data on the levels of microorganisms in various food products. Those data can be viewed as industry-wide process capability studies that provide a measure of the mean and variability of the foods produced by the industry. Such studies can be used to establish microbiological criteria and then, subsequently, to measure the effectiveness of industry performance and regulatory control policies and practices.

13.4.1 Establishing Criteria and Monitoring Procedures

Some of the general principles that determine which process evaluation techniques are most appropriate are introduced below.

Statistical process control methods allow the processor to control the mean and minimize the variability of each important process control parameter. If the process has achieved a predictable repeatability, the mean and the variability should remain relatively stable over time. In HACCP, SPC can be used to determine a process' capability to maintain its mean and variability in relation to process monitoring and verification analyses. When control charted (see Fig. 13.1, verification data provide a temporal history of how well the system has been controlled. First introduced in 1924 by W. A. Shewhart, control charts are the primary tool for visualizing, comparing, and analyzing process data. Accordingly, statistical concepts related to SPC will be discussed mainly in terms of these charts.

The stringency of a process control system is established by "control values" for parameters in the process or product that require intervention to maintain control. In HACCP, the control values associated with CCPs are critical limits and are used to separate acceptability from unacceptability (CAC 1997). The mean value of the control chart is set by the process average and in itself is not a control value in the sense of aforementioned CCPs; this is a subtle but notable difference and needs to be remembered in the present discussion of control charting (see the aforementioned note about the term "monitoring"). Product produced during a process deviation would be unacceptable to release until it could be determined that the loss of control did not result in food of unacceptable or uncertain quality and safety. In some cases if the process deviation exceeded the critical limit the process would be stopped and an investigation as to the root cause of the deviation performed.

The stringency of a food safety system is a relative measure and establishment of a control value is a decision that requires consideration of risk and of the consequences of a system failure. If a value is set in such a way that even minor changes in the mean or the variability will violate the established control value, then the system is very stringent. Conversely, if substantial deviation from the mean or a substantial increase in the variance is tolerated without an intervention, then the system is not stringent. Statistical process control charts reflect system performance and can be used to compare to mean values related to CCP values. A discussion of some general concepts is often helpful in providing a framework within which the individual approaches can be interpreted.

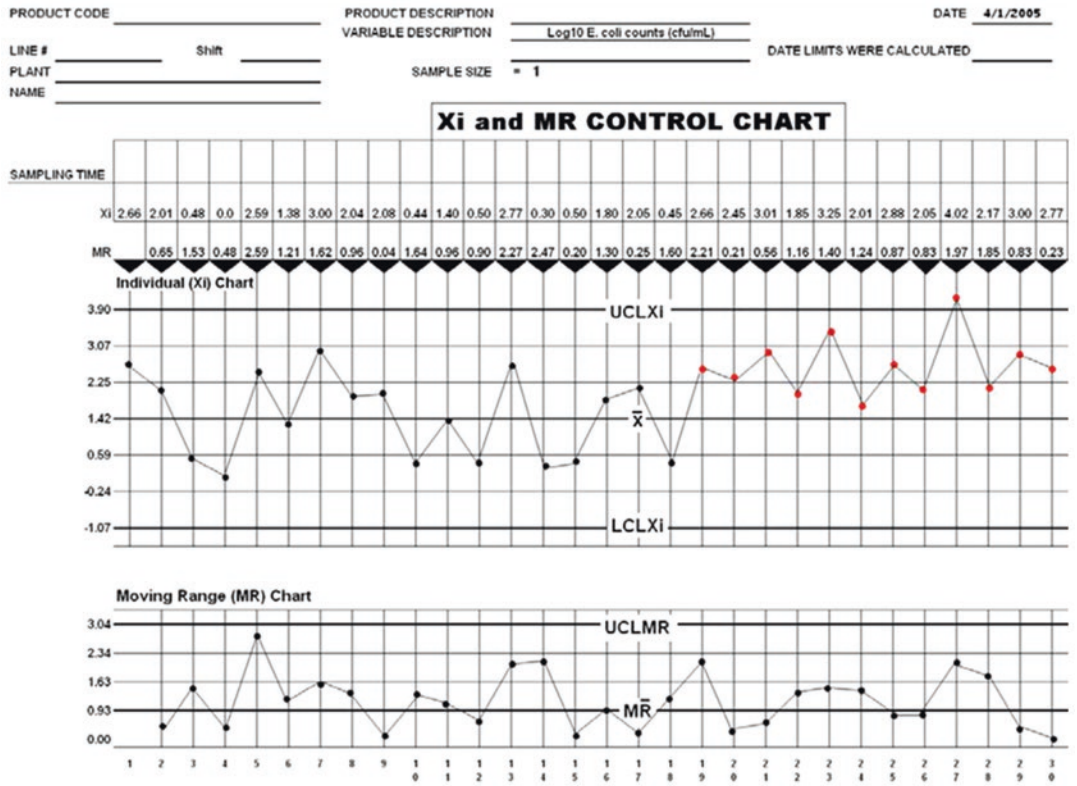


Fig. 13.1 Demonstrates a process with a positive shift in *E. coli* counts. At about point number 19 the process showed a positive shift. This was identified after the eighth consecutive point above average on the Xi chart, and confirmed by the out of control point exceeding the UCLXi on point number 27. Although the process average had shifted up there is no indication that the variation had increased. If this were the case, then the reason for the out of control pattern would be systemic, affecting the processing within the plant, and would not be from a source which would only affect a portion of the process output, such as a supplier effect. That is, certain possible causes could be eliminated from consideration

13.4.2 Types of Variability and Error

Two sources of variation are associated with food processes. The first is referred to as "common causes" of variation inherent in a process when it is operating as intended, i.e., "in control." One might think about common cause variation as that variation which will always be there (inherent to the process, e.g., mixing equipment); reducing the common causes of variation, while difficult to do, would improve the process. The second source of variation is referred to as "special causes" or "assignable causes", (e.g., change of food matrix, source of ingredients, change of season) of variation. This variability can occur when one or more steps in a process are no longer operating as intended (i.e., is out of control). In this instance, identification and correction of the special (assignable) cause of variation returns the system to the original degree of variability within which it was designed to operate. Eliminating these assignable causes of variation will have significantly more impact on the process than reducing common cause variation. Assignable variations often are related to issues associated with the reliability of equipment, personnel or ingredients.

Differentiating common and special causes of variation is usually achieved by comparing series of measurements that indicate whether the products meet the specifications previously established when the process was under control. In its simplest form, the results of such a set of measurements fall into four categories:

(a) The measurement correctly identifies the system as "in control,"
(b) The measurement correctly identifies the system as "out of control,"
(c) The measurement incorrectly identifies the system as "out of control," or
(d) The measurement incorrectly identifies the system as "in control."

Examples (c) and (d) above are referred to as Type I and Type II errors, when the measurements lead to incorrect interpretations. A Type I error (c) indicates that a process is out of control when, in fact, it is still operating in control. Such a Type I error can be viewed as a false alarm. Conversely, a Type II error (d) occurs when a series of measurements indicate that a system is in control when in fact it has an assignable cause of variation. The stringency of the criteria that indicate the emergence of a "special cause" of variability is based on the consequences of making Type I versus Type II errors.

13.5 Monitoring and Verifying a Single Lot of Food: Using SPC during Production

Two types of data are collected during the HACCP implementation, the data from monitoring CCPs and the data from verification activities. Control chart methodology is ideally suited to monitoring CCPs and other control points in an objective and consistent fashion. Decisions reached using control charts can be made with a measured degree of confidence. If the risk associated with a hazard is high, the control chart is a useful tool to assist in determining the chances of the process going out of control. If the measurement is instantaneous e.g., temperature, control is active, rather than passive, with control charts following the progress of a lot of food while it is being manufactured, detecting impending problems and allowing adjustments to be made before control is lost.

The necessity of quick feedback as a food is being processed generally precludes the use of microbiological tests in control chart applications related to HACCP monitoring. Thus, control charts are normally used to record physical or chemical measurements. Control charts are easily produced when automatic in-line measuring is used (e.g., recording temperature during milk pasteurization). Statistical control charts can be used in conjunction with industrial process control techniques so that when a critical limit is not met, milk is diverted by an automatic flow diversion valve to a holding tank, for re-pasteurization after the equipment has been adjusted to the required operating temperature. These non-microbial measures can still be good indicators of overall process monitoring and can, in turn, lead to important information about the food production process.

Another example of control charting is in recording the internal temperature of beef roasts during cooking. To achieve a performance criterion of a 6 $\log_{10}$ reduction of salmonellae it would be necessary to cook to an internal end point temperature of 62.8 °C and remain at this temperature or higher for a minimum of 4 min, these data can easily be charted, particularly if they are recorded with in-line devices. Other time-temperature combinations could be used to meet the same performance criterion (6 $\log_{10}$ reduction of salmonellae). To ensure the entire lot meets the performance criterion, internal temperature must be measured in the coldest area of the largest roasts in the lot. In addition, distribution of heat throughout the oven should be periodically verified (e.g., quarterly, monthly) to ensure the roasts placed on the bottom, middle and top layers of the cooking racks and in different areas of the oven meet the criterion. All of these measures could be control charted and followed as part of process verification.

Because continuous monitoring of the internal temperature of a number of roasts is even beyond the capabilities of many processors, many operators establish process criteria for the cooking conditions (i.e., oven temperature, humidity, time) as a simpler, cheaper, more convenient means to monitor the process. It is also common practice to limit the weight range and size of the roasts within the lot to avoid over-cooking and yield loss among lighter weight roasts. Frequent measurements of the oven

temperature can be collected automatically from thermocouples strategically placed throughout the oven. The data for the oven can be recorded automatically onto a chart computer-based logger that provides a visual record as the lot is being cooked. The oven operator can examine the chart to determine whether adjustments are necessary to meet the critical limit of 62.8 °C. Additionally, these temperature data can be control charted to statistically assess overall oven performance.

An alternative approach to meeting the performance criterion could be based on the total lethality for salmonellae that occurs during the heating and chilling cycle. For example, this could involve placing thermocouples into the roasts and documenting the time the internal temperature is at 57, 58, 59, 60 °C, etc. during heating and chilling. The cooking procedure could then be based on the incremental lethality that occurs at the times and temperatures above 57 °C, the sum of which would meet the 6 $\log_{10}$ reduction performance standard for salmonellae. This approach should be continuously monitored with thermocouples placed in the largest roasts to provide a temperature profile that can be used for verification. The accumulated time could be control charted as a measure of process verification.

It is important to note that biased sampling is used in the selection of roasts to be monitored. Here, biased sampling, i.e., information about the largest roasts and, in some cases, the location of the roasts within an oven is used to ensure all the roasts meet the criterion. If any of the selected roasts have not met the established criteria, then the entire lot must continue to be cooked. While information about variability is very important when establishing the cooking and monitoring procedures, information about both the mean and variability will determine the quality of the product and profitability of the process. In this example, and in many other food processes, a statistical evaluation is not used to decide when each individual lot is cooked. Statistical evaluation, however, can be an important tool in the design and validation of a process or part thereof.

13.6 Organizing Data from Across Multiple Lots of Food to Maintain or Improve Control During Production

This section also applies to individual lots of food, but primarily considers the organization and interpretation of data collected from multiple lots produced over days, months or even years, to develop a SPC program, enhance control of production and provide information necessary for continuous improvement.

The emergence of industrial process control programs such as HACCP has led to a substantial shift in the intent of microbiological testing programs. While testing of individual lots of food still occurs, increasingly industry and control authorities are focusing their testing programs on verifying that food control systems are effective (Buchanan and Schaffner 2015).

While many of the microbiological assays employed for these two approaches are virtually identical, the statistical tools and assumptions that help in interpreting the results of monitoring and verification differ from those for batch testing. Particularly for verification testing, data from multiple batches, often over extended periods of time, are evaluated. Thus, unlike batch testing, verification requires consideration of within-batch and between-batch variability.

It is important to re-emphasize that the purpose of such testing is not approval for release of batches or characterization of particular lots of product. In the case of HACCP systems, individual batches are characterized by monitoring CCPs, not verification testing. Instead, the purpose of such periodic testing is to provide:

- assurances that the conditions that enable a food process to produce safe products are being maintained

- a basis for analyzing performance trends so that corrective actions can be taken before loss of control
- insights into the cause for loss of control (e.g., periodicity of contamination)
- a warning that conditions have changed sufficiently such that the original HACCP plan may need to be reviewed/revised.

One of the most important features of control charts is that they allow visual tracking of results over multiple lots. As trends develop, not only does it become apparent when action is required, but it is also possible, by careful study of the data, to determine approximately when the trend started. Cumulative sum (Cusum) charts, which use the same data but plot it in a cumulative fashion, provide a clearer visual display for this purpose. MSUM (from moving sum) charts are another means of presenting cumulative data (Montgomery 2009). Knowledge of when a non-random influence entered the process can yield valuable clues to the identification and subsequent elimination of the problem.

It is not good practice to wait until a control chart indicates an out-of-control situation before taking action. There are several different "rules" for evaluation of control charts (Montgomery 2009) and organizations may adopt a portion (or all) of these rules. It is imperative that each process owner develop the type of evaluation system (i.e., Out of Action Control plan) and follow it. Some control charts have warning limits inside the control limits, to signal impending loss of control. Cusum charts are especially useful for early detection of trends. Many processes can gradually go out of control, providing plenty of warning as this occurs. Control devices may drift, mechanical components will wear (causing increasing variability as they do so), cleaning and disinfection procedures may be less stringently performed, or some change might occur in the ingredients. Sometimes even an abrupt change in some procedure may result in a gradual change elsewhere.

The development of upper and lower control limits is the basis of statistical process control. Even so, in many situations, the need for both upper and lower control limits may not be readily apparent. One-sided process charts could be constructed for situations where the upper limit is of concern. Examples include the pH of an acidified food that is to be distributed at ambient temperature or the retort temperature for a canned food that must be kept above a certain minimum requirement, but too high a temperature is wasteful of resources or leads to process-derived contaminants. Frequently at CCPs in a food process, one limit controls product safety while the other limit represents economy of manufacture or some aesthetic feature of the food. These type of industrial process charts are in many cases as useful as SPC charts for assessing or optimizing process control.

In addition to monitoring existing, well-established processes, control charts are useful in modifying/improving existing and developing new processes. By their nature, control charts draw attention to unusual results that have occurred due to the influence of some non-random and controllable factor. Hence, in process design, control charts can be used to help isolate sources of process variation so that these sources can be traced and, if possible, eliminated.

Acquiring data in this manner can provide important insights concerning the performance and reliability of a food safety system and the types of problems encountered. As a means of demonstrating this point, Figs. 13.2, 13.3, 13.4, 13.5 and 13.6 depict graphic examples of hypothetical microbiological data acquired to verify performance of a food safety system in relation to an established acceptance criterion.

Figure 13.2 depicts a system that is under control. Even with a well-controlled system deviations characteristic of the system occasionally occur. Setting the criterion for what is or is not a "failure" is dependent on the level of performance that can be expected (i.e., a process's variability) and achieved (i.e., technological capability), and the consequences of not meeting the acceptance criterion. In a well-controlled processing system, the majority of data tend to cluster around a central value.

Figure 13.3 depicts the same system with greater variability. This is reflected in an increased number of samples above the acceptance criterion and an increase in the scatter of values that meet the acceptance criterion. Such a scenario could indicate one or more factors not being controlled.

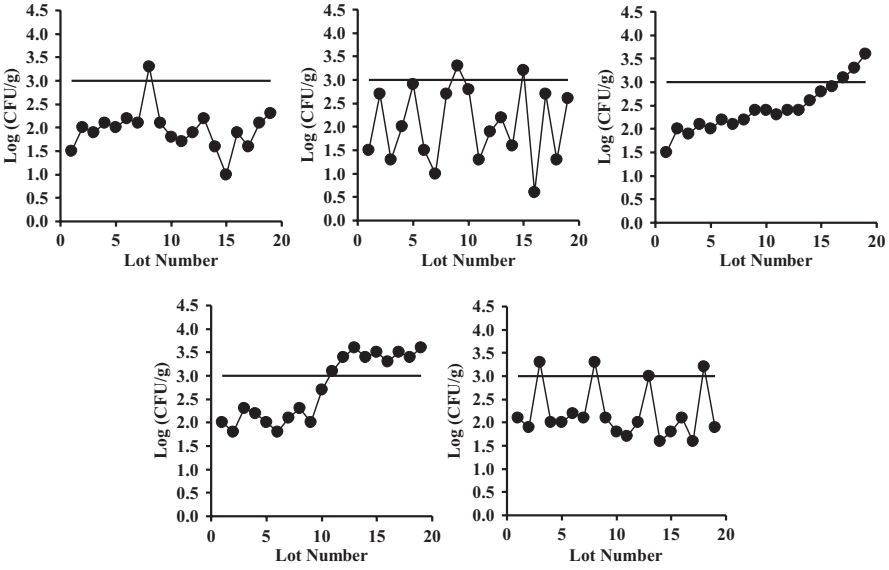


Fig. 13.2, 13.3, 13.4, 13.5 and 13.6 Hypothetical examples of using data from an assay for a microbiological indicator to verify the effectiveness of a food safety system. Examples depicted include the system under control (Fig. 13.2) - *upper left*, lack of control due to excess variability (Fig. 13.3) - *upper center*, loss of control due to gradual (Fig. 13.4) - *upper right* and abrupt (Fig. 13.5) - *lower left* process failures, and loss of control due to a reoccurring, transitory failure (Fig. 13.6) - *lower right*. The solid horizontal line depicts a hypothetical microbiological criterion above which a sample is considered only marginally acceptable. A criterion based on the presence of more than a specified number of marginal samples within a specified period of time would be the basis for determining if a process is out of control and requires corrective actions (Adapted from Buchanan (2000))

Figure 13.4 depicts a situation where a component of the process is losing its effectiveness over time. It is apparent, through trend analysis of the data that it might be possible to detect and correct this deficiency before the acceptance criterion is exceeded. This trend indicates a gradual loss of control at an important processing step. An example of this type of failure might be the buildup of cooked egg on the holding tubes of a liquid egg pasteurizer, leading to decreasing effectiveness of the heat treatment. By comparison, Fig. 13.5 depicts an example of a loss of control such as when a key piece of equipment failed abruptly.

Finally, Fig. 13.6 provides an example of a system with distinct periodicity. Such data indicate the existence of a recurring, intermittent problem. This pattern can occur with a seasonal effect, if the data were to represent a summary chart spanning years, or lots produced on Mondays reflecting microbial growth on inadequately cleaned equipment over the weekend.

While many food producers use microbiological testing to establish microbiological profiles for their products, these data are often not analyzed and evaluated in a rigorous manner. The knowledge that can be gained from these data can be greatly enhanced through use of the relatively simple statistical tools that are described in this chapter. Considering the cost incurred in conducting microbiological testing, the use of these statistical tools should be viewed as value-added, increasing the manufacturer's return on their investment in testing.

13.6.1 Variables Charts of Quantitative Data

As mentioned above, control charts are the primary means for arraying process control data for visualization and analysis. However, the type of control chart used will depend on the type of data being evaluated. As discussed in Chap. 7, microbiological analyses generate two types of data, attribute data

(non-quantified, presence/absence assays) and variable data (quantitative population density determinations).There are different classes of control charts for evaluating those data. Attribute charts will be discussed in Sect. 13.6.8.

13.6.2 General Principles of Variable Control Chart Construction

Statistical process control charts are plots of data over time (Montgomery 2009; Ryan 1989) and may represent data from one lot or from across multiple lots. The x-axis on the control chart is usually the time at which the sample or samples were collected during the process, and the y-axis is the value obtained for the measurement. The control chart consists of three parallel lines: a lower control limit (LCL), a center (or central) line, and an upper control limit (UCL) (Fig. 13.7). In some instances where the lower control limit is below the lower limit of detection (i.e., absence), the LCL is assumed to be zero or some pre-designated value below the lower limit of detection.

Each step in a process has a degree of inherent variability. When combined, the variability of each step contributes to the overall variability of the system. In a well-controlled system, data points tend to cluster around a central value (centerline as given above). Traditional statistical measures of central tendency include the mean, median, and mode, and the measures of variability such as the range, standard deviation, and standard error of the mean. The centerline in a control chart typically is a measure of central tendency, whereas the flanking lines are limits based on the calculated degree of variability.

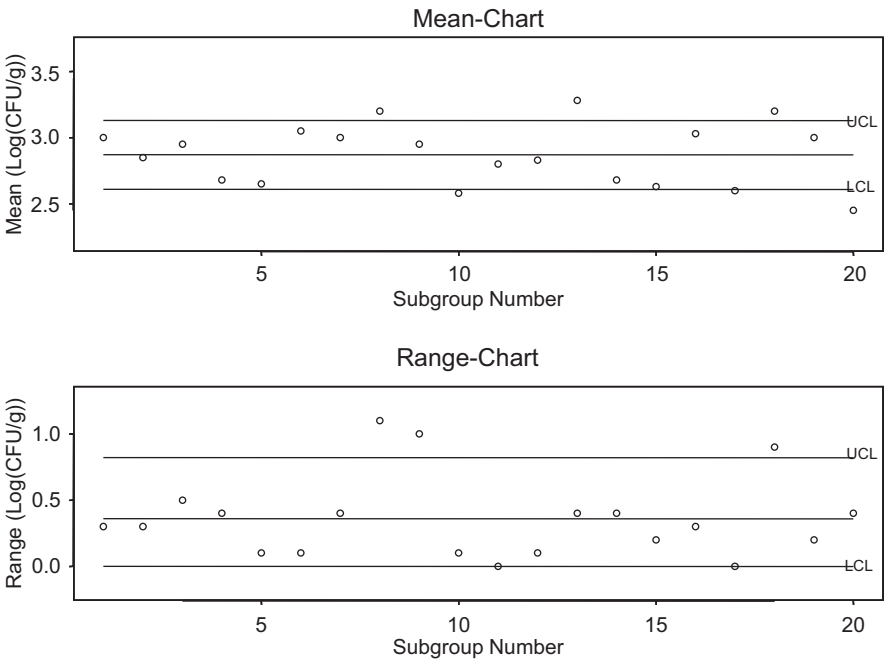


Fig. 13.7 Hypothetical example of $\bar{X}$ and R control charts developed using a process capability study that measured the "Total Aerobic Plate Count" for 20 data subgroups each with 4 replicates (Table 13.1). The middle solid line represents the "target value" ($\bar{\bar{X}}$ or $\bar{R}$) and the flanking dotted lines represent ± 3σ

To appreciate how the LCL and UCL are often established, it is necessary to understand the "sigma" (σ) concept as it applies to control charts. The σ concept is similar to that used in describing the variability of batches or lots. Control charting generally assumes the distribution of data collected during the process is Normal or approximately Normal. Based on the normal distribution, approximately 68% of values will fall within plus or minus 1 standard deviation of the mean; approximately 95%, within 2 standard deviations of the mean; and approximately 99.7%, within 3 standard deviations of the mean (Fig. 13.8). In this way, "one sigma" (1σ) refers to 1 standard deviation from the mean; "2 σ" to 2 standard deviations from the mean; and "3 σ," to 3 standard deviations from the mean. Control limits are most commonly set at plus or minus 3 σ from the mean. When 3-σ control limits are used, the probability of any particular datum point being outside the control limits by chance alone is 0.3% when the process is actually in control. Thus, if the frequency at which values fall above or below 3 σ is greater than 0.3%, then the process is deemed to be out of control This is one of the basic rules of control charting as developed by Shewart in the 1920s and these rules are called the "Western Electric Rules" (Montgomery 2009). Whether or not the process owner decides to take an action due to the out of control state is dependent on the OCAP.

In addition to determining when a process is out of control, control charts and their measures of central tendency and variation can be used to predict the frequency that failures will occur despite a process being in control (i.e., frequency of type I errors). For example, Peleg and co-workers (Nussinovitch et al. 2000; Peleg et al. 2000) used control charting in combination with a probabilistic model to predict the frequency of unusually elevated bacterial counts in foods.

As a means of introducing general concepts related to process control charting, the $\bar{X}-R$ control chart will be used as an example. "$\bar{X}-R$" denotes the use of the mean ($\bar{X}$) of a subgroup of samples and the range (R) between the smallest and largest value in the subgroup of samples. It is ubiquitously used in variables charting. The $\bar{X}-R$ chart is actually two charts with one being a measure of central tendency between subgroups (i.e., the mean, $\bar{X}$ chart) and the other a measure of variability within subgroups (R chart). It is based on the comparison of small subgroups (typically, n < =10), thus fulfilling the original intent or precision of the Normal distribution, i.e., comparison of means. $\bar{X}$ and R are usually included on the same control chart to facilitate their use in tandem to establish whether the process is in control. The $\bar{X}-R$ chart is one of the most useful from a practical standpoint because it

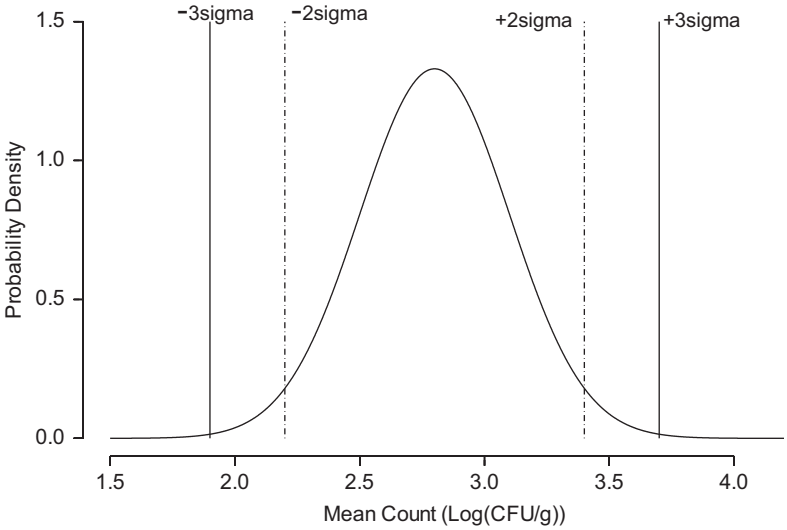


Fig. 13.8 Density of a normal distribution

is simple to construct and simplest to understand. The steps for constructing a basic $\bar{X}R$ control chart are presented below.

The first step in developing an $\bar{X} - R$ chart is to define a subgroup, frequently a set of results from verification samples or monitoring data that are collected during a short time when the process is known to be under control. The variable of interest is measured for each item in the subgroup. Before the control chart is constructed, the general recommendation is to collect data on 20–40 subgroups, with each subgroup consisting of samples (n) of 4–5 units per subgroup (Montgomery 2009). As an illustrative example, a set of hypothetical total aerobic plate count (TAPC) data taken to verify the microbial quality of a ready-to-eat food when all manufacturing processes are "in control" is provided in Table 13.1. In this example, the control limits are based on 3 σ and an equal number of items in each subgroup; here the subgroup is n = 4.

1. Calculate the mean ($\bar{X} = [X_{1 + \ldots +}X_n]/n$) for each subgroup (Table 13.1, Mean column). If evaluating microbiological population density data, the $\log_{10}$ of the individual values are used, thus converting the Log normal distribution associated with microbial "concentrations" to a Normal distribution of log values.
2. Calculate the range (R) for each subgroup (Table 13.1, Range). The range is the difference between the maximum and minimum value of items in the subgroup.
3. Compute the mean range ($\bar{R}$) for all subgroups. The mean range is the sum of the ranges for all of the subgroups divided by the number of subgroups (n_{sg}). In Table 13.1, $\bar{R}$ value is 0.36.

Table 13.1 Hypothetical results of a process capability study for a ready-to-eat food wherein total aerobic plate count data ($\log_{10}$ cfu/g) are used to verify the acceptability of a product

Subgroup	Sample 1	Sample 2	Sample 3	Sample 4	Mean, $\bar{X}$	Range, R
1	3.1	3.0	2.8	3.1	3.00	0.3
2	2.7	2.9	3.0	2.8	2.85	0.3
3	2.8	3.3	2.8	2.9	2.95	0.5
4	2.9	2.5	2.6	2.7	2.68	0.4
5	2.6	2.7	2.7	2.6	2.65	0.1
6	3.1	3.0	3.0	3.1	3.05	0.1
7	3.2	2.9	2.8	3.1	3.00	0.4
8	3.8	2.8	2.7	3.5	3.20	1.1
9	3.5	3.0	2.5	2.8	2.95	1.0
10	2.6	2.5	2.6	2.6	2.58	0.1
11	2.8	2.8	2.8	2.8	2.80	0.0
12	2.9	2.9	2.7	2.8	2.83	0.1
13	3.0	3.3	3.4	3.4	3.28	0.4
14	2.9	2.5	2.7	2.6	2.68	0.4
15	2.5	2.6	2.7	2.7	2.63	0.2
16	3.1	2.8	3.1	3.1	3.03	0.3
17	2.6	2.6	2.6	2.6	2.60	0.0
18	2.8	3.4	3.7	2.9	3.20	0.9
19	2.9	3.0	3.1	3.0	3.00	0.2
20	2.4	2.3	2.7	2.4	2.45	0.4
$\bar{\bar{X}}$					2.87	
$\bar{R}$						0.36

Table 13.2 Parameters for Determining "3-σ" Control Limits for X-bar and R Charts (Montgomery 2009)

Sample Size (n) per Subgroup	A_2	D_3	D_4
2	1.880	0	3.268
3	1.023	0	2.574
4	0.729	0	2.282
5	0.577	0	2.114
6	0.483	0	2.004
7	0.419	0.076	1.924
8	0.373	0.136	1.864
9	0.337	0.184	1.816
10	0.308	0.223	1.777

4. Compute the grand mean for all subgroups. The grand mean ($\overline{\overline{X}}$,"x-double-bar") is the sum of the means of all the subgroups divided by the total number of subgroups ($\overline{\overline{X}} = [\Sigma \overline{X}\,]/ n_{sg}$). In the current example, $n_{sg} = 20$ and the $\overline{\overline{X}}$ value is 2.87.
5. Set the centerline of the $\overline{X}$ chart at $\overline{\overline{X}}$, and set the centerline of the R chart at $\overline{R}$ (Fig. 13.6).
6. Calculate the 3-σ control limits around the target value for the $\overline{X}$ chart. The lower control limit (LCL) is equal to [$\overline{\overline{X}} - (A_2 \bullet \overline{R})$]. The upper control limit (UCL) is equal to [$\overline{\overline{X}} + (A_2 \bullet \overline{R})$]. For small subgroups ($A_2 \bullet \overline{R}$) is an estimate of 3-σ. The value of A_2 can be obtained from Table 13.2 for subgroups with sample size (n) of 2–10. In the current example the LCL = 2.61 and the UCL = 3.13.
7. Calculate the control limits for the R chart. The lower control limit (LCL) is equal to $D_3 \bullet \overline{R}$. The upper control limit (UCL) is $D_4 \bullet \overline{R}$; here the value of $D_4 \bullet \overline{R}$, is an estimate for 3-σ. The values of D_3 and D_4 can be obtained from Table 13.2 for subgroups with sample size (n) of 2–10. In the current example the LCL = 0.00 and the UCL = 0.82.
8. Plot the $\overline{X}$ values on the $\overline{X}$ chart and the R-values on the R chart (Fig. 13.7). Plotting the current example makes it apparent that there is a fair degree of variability associated with both the central tendency ($\overline{X}$ chart) and variability (R chart) associated with this hypothetical process.

As indicated earlier, the $\overline{X} - R$ chart is one of the simplest variable control charts. Detailed descriptions of the procedures and uses of the wide variety of other variable control charts are available from standard references such as ASTM (1990) and Duncan (1986). These procedures can be easily handled in spreadsheet software and there are a variety of statistical analysis packages that offer SPC options that one may use as well (e.g., Minitab™, JMP™, Systat™, etc).

13.6.3 Corrections When Data Are Auto-Correlated

As discussed previously, an underlying assumption in the development of most control charts is that, as the individual data points are plotted, they are randomly distributed according to a Normal ($\log_{10}$ values) or Lognormal (arithmetic) distribution over time. However, in actual production settings, successive measurements of product parameters collected sequentially over time are often correlated with one another (i.e., the measurements are "auto-correlated"). Auto-correlation is more likely when values are collected close to one another in time. Auto-correlation affects the pattern of data on control charts, with implications for setting control limits. In some cases data auto-correlation can affect overall system control. Standard SPC references (see Montgomery 2009; Wheeler 2010; Levinson 2011) provide in-depth coverage of auto-correlated data and control charts. These references provide suggested methods to address auto-correlation of data used in control charts. A standard practical approach is to use overall batch means as a method to overcome auto-correlation bias

(Montgomery 2009). Depending on particular circumstances, other methods to reduce autocorrelation should be examined.

13.6.4 Special Considerations for Charting Individual Counts of Microbial Population Density

Data on concentrations of pathogens or indicator organisms can be presented in simple variables charts such as the hypothetical example in Fig. 13.9. This example shows numerical estimates of concentrations of indicator organisms in samples of a finished product. This chart shows typical fluctuations in viable counts up to week 40. After week 40, the same sort of fluctuations occur, but around a much lower midpoint. This could reflect a change in raw materials or equipment, a seasonal effect, a change in the analytical method, etc. Even though the change appears positive, investigation is warranted since it could represent either an analytical problem or a means for identifying a factor that could consistently enhance the performance of the system. The C chart in Fig. 13.10 is used when sample size (number of units or amount of material, being sampled for one analysis) is constant for all samples. (see Appendix F.1.2, (AOAC 2006) for further details on this type of chart).

One of the most straightforward means of considering a set of quantitative microbiological data is to chart the individual test results. Such a control chart, commonly called an individual measurements chart (I or X_i chart)," is simple and rarely misleading. This chart is presented as Fig. 13.1. Unusually high counts, or patterns of high counts, are typically the criteria used to designate loss of control and raise safety concerns. Conversely, unusually low counts, or patterns of low counts, may reflect a potential process improvement or a need to review analytical protocols. Obvious trends of either sort would merit attention. Subtle trends in the values for an indicator should not necessarily be viewed as trends at all, since some drifting up and down of indicator levels over weeks or months is to be expected and might be considered random variation. However, such trends may warrant a review of the process since they may be early warning of a gradual loss of process control (Figs. 13.4 and 13.5, and thus indicates the need for corrective action before loss of control occurs.

13.6.5 Selecting Limits for Variable Control Charts of Microbial Counts

Generally the control limits are set at +/− 3 σ and there are SPC procedures that can help the initial choices of limits. The following steps will provide a reasonable starting point. One of the key factors in selecting the limits is the test results for which no microorganism was detected, i.e., test value = 0.

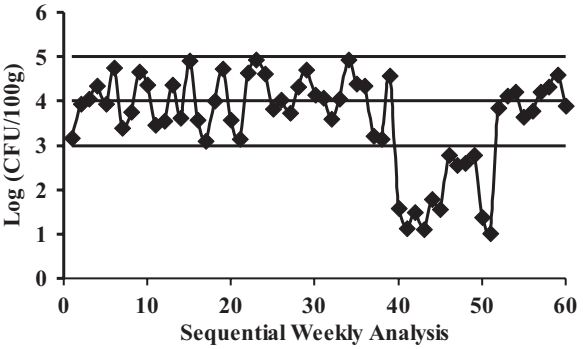


Fig. 13.9 Hypothetical example of a "variables control chart" for a microbial indicator assay conducted weekly. The center horizontal line represents the mean value and the two flanking lines represent the upper and lower "warning" limits. An apparent period of significant improvement is depicted between weeks 40 and 51

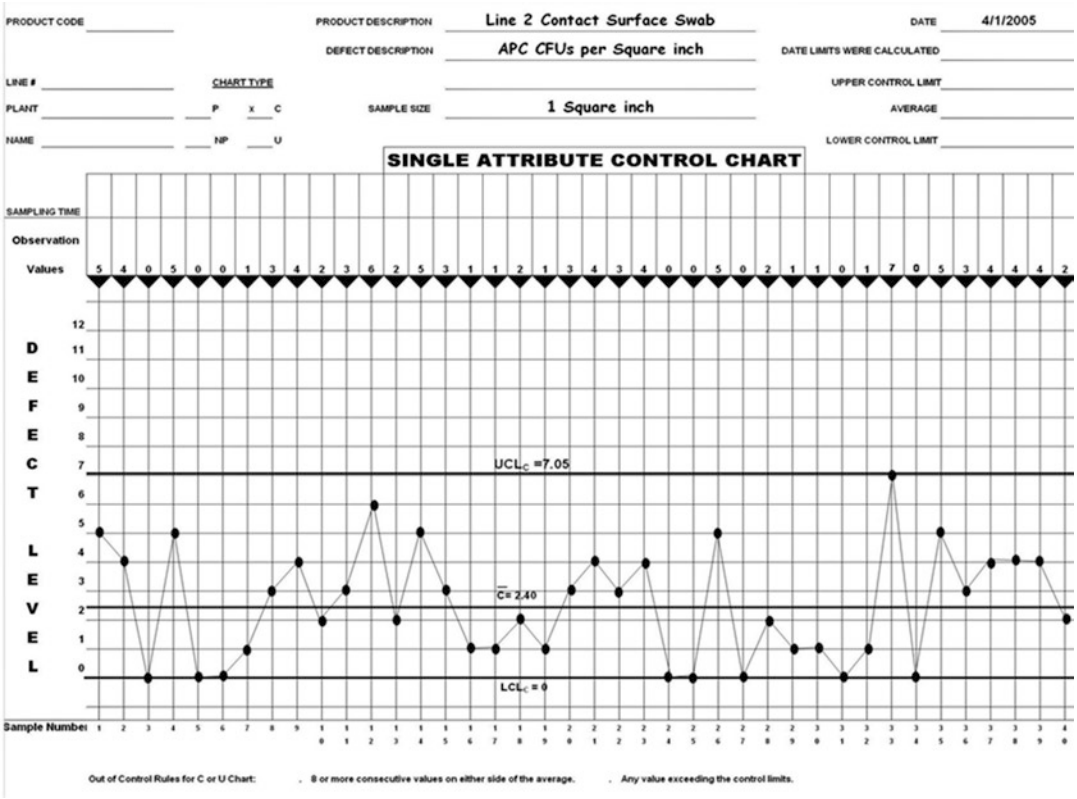

PRODUCT CODE
PRODUCT DESCRIPTION
Line 2 Contact Surface Swab
DATE
4/1/2005
DEFECT DESCRIPTION
APC CFUs per Square inch
DATE LIMITS WERE CALCULATED
LINE #
CHART TYPE
UPPER CONTROL LIMIT
PLANT
P x C
SAMPLE SIZE
1 Square inch
AVERAGE
NAME
NP U
LOWER CONTROL LIMIT
SINGLE ATTRIBUTE CONTROL CHART
SAMPLING TIME
Observation Values
5 4 0 5 0 0 1 3 4 2 3 6 2 5 3 1 1 2 1 3 4 3 4 0 0 5 0 2 1 1 0 1 7 0 5 3 4 4 4 2
DEFECT LEVEL
12 11 10 9 8 7 6 5 4 3 2 1 0
UCLc =7.05
c̄= 2.40
LCLc = 0
Sample Number
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
Out of Control Rules for C or U Chart:
. 8 or more consecutive values on either side of the average.
. Any value exceeding the control limits.

Fig. 13.10 A C chart showing the 40 data points of the baseline data plotted

However, such a value is not directly useful for two reasons. First, the need to convert microbial population density data to $\log_{10}$ values results in the 0 values being undefined (and zero values are ignored by some software). Second, the inability to detect a microorganism in any specific sample reflects two possibilities: that the microorganism truly is not there, or that the microorganism is present, but at levels that the sampling protocol or analytical methods are incapable of detecting.

Consider a situation where at least 90% of the test results are expected to be quantifiable above the lower limit of detection for the method employed. Any results below the limit of detection (i.e., no microorganisms are detected), are assigned a value of one half of the limit of detection, and transformed into a $\log_{10}$. (This might slightly underestimate the standard error. This would make the resulting "warning rules" and "stopping rules" slightly over cautious, but this can be adjusted when a large number of test results are available.) The limit-setting study would begin with testing at a high frequency to acquire enough results to study their average and their variability. Typically 30 or more would be acquired and charted. The mean ($\overline{X}$) and the standard deviation (σ) are computed, and "warning limits" are set at $\overline{X} \pm 2\sigma$, and upper and lower "stopping limits" are set at $\overline{X} \pm 3\sigma$.

When more than 10% of the limit-setting data are "zero values (i.e., too low to be quantified), it is better initially to derive the warning and stopping limits by comparing the gap between percentiles of the test data with that which would be predicted by the normal distribution. If less than half of the results are below the lower limit of detection (i.e., zero), a simple approach is to find the difference between the $\log_{10}$ of the 50th percentile (i.e., 50% of the samples have a lower value) and the $\log_{10}$ of the 75th percentile. With a normal distribution, this gap in the $\log_{10}$ values should cover 67% of the σ. Dividing the gap value 0.67 provides an estimate of σ. The 50th percentile value is used in place of

$\bar{X}$ in drawing the limit lines. Using these estimates of $\bar{X}$ and σ, the limits for the control chart are drawn as described above.

If more than half of the results are below detection, consider whether to proceed with a variables chart. It might be more appropriate to use an attributes (presence/absence) chart. If that is not the preferred option, however, any table of cumulative probabilities of the normal distribution will provide the 50th percentile and σ from the gaps in the percentiles of the data. For example, the gap between the 70th and 90th percentiles should be 76% of σ. As a rough rule of thumb, percentiles greater than 90th should not be used for this purpose.

Once the initial limits have been established, the first test results acquired subsequently should be scrutinized carefully as a means of assuring that the data used to set the initial limits were acquired when the process was in control. Ordinarily, one would hope not to find any values outside the above limits at this stage. If deviations are observed, then the data should be studied for any trend, and any trend identified should be investigated. If any result is below – 3σ or above +3σ, or if 3 of any 5 successive results are below – 2σ or above +2σ, investigate possible causes such as a change in the process or ingredients or a change in the sampling or analytical protocols. In such circumstances, the process capability study will have to be re-run with the new data until the warning and stopping limit values stabilize. When the process capability study has been completed without any deviations or apparent trends, then these limits at $\bar{X} \pm 2\sigma$ and $\bar{X} \pm 3\sigma$ should be selected for initial control limits for verification tests.

As verification testing proceeds, if it appears that the limits selected are giving an unreasonable number of "false alarms," (which is typically specified in the OCAP) then the limits should be compared against the likelihood of type II errors (i.e., not catching a safety defect) and expanded if appropriate. Such an expansion of the limits should be done cautiously with continued or increased scrutiny until sufficient data are acquired such that the process can be adequately judged in relation to:

- how truly rare will deviations be when the process is in control, and
- how frequently control is lost.

Two examples of reasons why the initial limit-setting exercise might have proved too restrictive are:

- Seasonality might cause $\bar{X}$ or σ to shift in ways that will make the limits too tight. If seasonality has no effect on $\bar{X}$, then it will probably have no effect upon σ. If it does affect $\bar{X}$, then (a) the charts may need to be adjusted by drawing seasonal $\bar{X}$ lines on them, and (b) the data must be studied to see if the σ has also changed by examining the R or s chart. The seasonal changes in $\bar{X}$ and in the control lines can define a series of zones with different straight lines or $\bar{X}$ can be fit by a curving line with σ-determined lines curving at fixed distances above and below $\bar{X}$. However, if seasonality produces an unacceptable risk to safety, the appropriate response would be to eliminate the variability due to seasonality, and not to modify the control chart limits.
- If the limit-setting study was done with a single source of raw materials and the process then proceeds to use a variety of sources, an additional source of variation has been added, and the initial control limits will be affected. When more data, covering more sources of raw materials, are available, $\bar{X}$ and σ should be recalculated. Again, if the additional sources of raw material represent an unacceptable risk to safety, the appropriate response would be to eliminate the unacceptable variability associated with the new source(s) of raw material, and not to modify the control chart limits.

13.6.6 Caution in Interpreting Certain Types of Variables Charts

"Moving Range" (MR) charts are useful for process control monitoring of parameters such as pH and temperature. These charts track the absolute value of the difference between each result and the previous one. Declines and increases are treated the same. When dealing with levels of microorganisms, a

significant decline in microbial concentration may not have the same significance as an increase. This is because downward trends are generally not a cause for alarm, and a low value preceding a high value looks significant as a range but doesn't make the high value any more important.

Standard procedures are described in general quality control texts for converting the average of the moving ranges into an estimate of σ. However, this is also not advisable for pathogens and indicator organisms. Weather, seasonal changes, shifting harvest zones, early or late crops, spot markets for ingredients, and other factors will force $\bar{X}$ and MR values up or down over periods of multiple tests in ways that cannot be predicted very precisely. This will induce a degree of auto-correlation. As described above, auto-correlation is the tendency for results to be more like those adjacent to them in test sequence and less like those farther away in sequence. For this reason, the distribution of differences between successive test results will usually underestimate the true variability of microbial levels in foods.

For similar reasons, cumulative sums of differences in the observed $\log_{10}$ microbial concentrations and some target log numbers) (denoted CUSUMs) are not appropriate for these variable charts. The mean, or target log number is not truly a target value, since one does not intend to achieve a target value of pathogens. In this instance, the uncontrolled shifting of $\bar{X}$ results in an inability to set standard limits for a chart of the mean of the previous n observations. However, such charts, for small n, would still be useful as a means of illustrating shifting patterns, as well as quantifying variability that might occur as a result of seasonal variation. (Note that CUSUMS do have utility for attribute charting.)

13.6.7 Other Control Charts that Can Be Used for Microbiological Data

When process counts provide estimates of levels of product (size, weight, voltage, number of organisms), the estimated levels can be considered as variables data and the classical control chart procedures can be used. There are several control charts that may be used to chart variables type data. In addition to the X-bar and R chart example above, other charts are: the Xi and MR, (Individual and moving range), CUSUM, (Cumulative Sum) and X-bar and s, (Average and Standard Deviation). This example includes the X_i and MR charts. The X_i chart just involves plotting the individual results over time. The MR chart involves a simple calculation of taking the difference between the present sample result, X_i and the previous sample result. X_{i-1}. Thus, the points that are plotted are: $MR_i = X_i - X_{i-1}$, for values of i = 2, …, n. These charts are chosen because they are easy to construct and are common charts used to monitor processes for which control with respect to overall levels of product measurement is desired (see Appendix 1 of AOAC (2006)).

The baseline data, when plotted, produce a "stable appearing" process. The limits are then transferred to a blank control chart and X_i and MR_i values are plotted as they are collected. After the X_i and MR_i are plotted and connected to the previous point with a straight line, both the X_i and MR charts are viewed for out of control sequences. Pyzdek (1989) suggests the following out of control rules be used:

X_i Chart:

1. Any point exceeding a control limit
2. Eight consecutive points on the same side of the average, (X)

MR Chart:

1. Any point exceeding a control limit
2. Eight consecutive points on the same side of the average, (MR)

Figure 13.1 demonstrates a process with a positive shift in *E. coli* counts. At about point number 19 the process showed a positive shift. This was identified after the eighth consecutive point above average on the X_i chart, and confirmed by the out of control point exceeding the UCL X_i on point number 27. Although the process average had shifted up there is no indication that the variation had

increased, (Fig. 13.1, MR chart). If this were the case, then the reason for the out of control pattern would be systemic, affecting the processing within the plant, and would not be from a source which would only affect a portion of the process output, such as a supplier effect. That is, certain possible causes could be eliminated from consideration.

13.6.8 Attribute Charts of Presence/Absence Determinations

One of the simplest, most widely used microbiological tests for specific pathogens or indicator organisms is presence/absence testing. An analytical unit of known weight (mass) is tested by a standard method to determine whether the microorganism of interest is, or is not, detected in that food sample. Over time, this type of microbiological assay can be used to assess the maintenance of food safety control systems. However, interpretation of such tests is highly dependent on the method used to determine the presence of the microorganism, particularly its lower limit of detection. Consequently, it is generally not possible to compare or combine the results from one data set with those from another unless both data sets were acquired using the same standard method that has consistent analytical performance characteristics.

Consider a hypothetical example of a single microbiological presence/absence assay for *Salmonella* performed once per day for a total of 50 successive days as a means of process verification. The microbiological test requires 48 h to complete so there is an effective 2.5-day delay between the sample being taken and the results becoming available. Figure 13.11 depicts these data as a simple control chart. Let us assume that when the process being evaluated is under control, about 1 of every 4 assays is positive for *Salmonella*. Further, the stringency of the system has already been established based on the criterion that if *Salmonella* is detected on four successive days, this is considered a loss of control due to the introduction an attributable source of variability (this would be outlined in the OCAP). In this example, we have included an abrupt failure of a critical part of the process on day 29 that is not detectable by normal process control monitoring procedures but detected by the supplemental verification testing. While evaluation of the control chart ultimately allows the user to estimate when the failure was likely to have taken place, it shows that there is a substantial delay before an action can be taken. Based on the established criterion of 4 consecutive positive assays, if the results were available instantaneously, the chart would indicate a loss of control at day 33. However, because of the 2.5-day delay in acquiring the data, the actual day when the loss of control would be acted upon would be day 37. This is reflected in the control

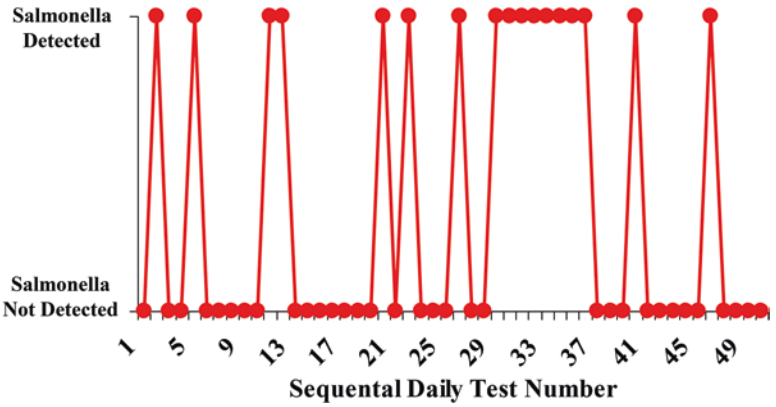


Fig. 13.11 Hypothetical example of an "attributes control chart" for a single daily detection/non-detection assay for *Salmonella* spp. microbe where in-control detection rate is 0.25 (25%). In this example, control is lost on Day 29 and restored on Day 37

chart (Fig. 13.11) when it returned to "normal" on day 37. It is apparent from this simple example that the "response time" associated with a control chart is based on the frequency of testing, the decision criterion established, and the time required to analyze the samples. For example, if the decision criterion had been 3 consecutive positive samples, the failure would have been detected a day earlier. However, this more sensitive criterion would increase the risk of Type I errors.

Presence/absence charts illustrate a sequential pattern of yes/no results. The usual assumption behind these charts is that when a process or system is operating under control there is some fraction "*p*" of sample units that will yield a "yes" result when analyzed. It is further assumed that successive test results are independent of each other. Analysis of charts shows whether these assumptions hold, and might lead to process improvements. For example, if the day shift has a higher *p* than the night shift at the same plant, this would suggest that there might be an additional source of variability during the day shift (an assignable cause) that may be reducible. Conversely, if one observes a longer than expected series of "no" results, the assumption of the independence of observations may not be true, and potentially could lead to identification of means for further process improvements.

If there is a change in a process that causes *p* to increase markedly, the frequency of detecting the microorganism of interest would be expected to increase (i.e., an increased frequency of "yes" results). Verifying that *p* is not changing is accomplished by tracking the sums of detection results over some interval of results. Starting from some beginning point in collection of data, one can determine for a number of future observations, *n*, an upper limit, denoted U(*n*), that should not be exceeded by the cumulative sum of the samples in which the microorganism is detected. For this reason, this statistic is called the "cumulative sum", or CUSUM. Standard statistical software included in most spreadsheet programs will compute the probability of getting *j* positive isolations out of *n* tests when the probability of the presence of the microorganism is *p* (a binomial probability). The tolerable degree of rarity needs to be decided before intervening in the process. Then that criterion will determine U(*n*) for every *n*. The process capability study that estimated *p* will be followed by creation of a table of values of U(*n*). Then after each test the CUSUM is compared with U(*n*). As long as CUSUM does not exceed U(*n*), the statistic has not shown a loss of control.

There are practical limits to the length of sequential results that are closely related to each other. The CUSUM is also fairly complex. For these reasons, another statistic is sometimes preferred. After the CUSUM has been followed up to a fairly large *n*, there is little loss of discrimination if the most recent (fixed) *n* results are summarized, without increasing *n* further. This number of times the presence of the microorganism was detected in the previous *n* results is called the "moving sum", or MSUM, because the count is derived from a zone that moves along the string of observations. The limit for this fixed MSUM is of course the same as that for the nth step in the CUSUM. If one selects a small *n* for evaluating the MSUM, one can achieve a rapid detection of any major shift in *p*. On the other hand, if *p* shifts slowly upward, the sum over a small *n* will not be sensitive enough to detect it. A larger *n* is more discriminating, and therefore is more sensitive to even gradual shifts in *p*, but a large *n* can delay the discovery of the shift. One of the MSUM techniques that has been used extensively with microbial food safety verification testing is the "Moving Window" assay (see below).

This dilemma between quick response and high sensitivity can be overcome by using multiple MSUMs. These are a compromise between a single MSUM and the CUSUM. For many processes, a choice of two MSUM charts, a short interval for responsiveness and a long interval for sensitivity, can be very useful. MSUMS, by their nature, are highly correlated with their predecessors, so apparent long strings of MSUMS moderately above the mean value have no special importance. Increased frequency of testing can be especially valuable when MSUMS or CUSUMS get close to their action limits. When these limits are exceeded, the situation changes to one of investigational sampling (see Chap. 9) and problem solving to determine the new source of attributable variability and return the process to an in-control state.

13.6.9 Other Examples of Using Attribute Control Charts

13.6.9.1 Control Chart for Poisson Distributed Characteristics, with One Sample Size – the C Chart

When counts are not high and there is a small probability of not detecting any colony forming units (recorded as ND), the counts seen on a plate cannot readily be considered as variable data, as shown in Sect. 13.6.5. In this case, a discrete distribution, such as a Poisson distribution or negative binomial distribution can be considered for modeling the distribution of counts (where ND is zero). Microbiological examples which fit Poisson-like probability distributions are not as common as those which lend themselves to the binomial or normal distributions. The Poisson distribution is characterized completely by the value of one parameter, which is the expected value of the distribution. The variance of the Poisson is equal to the expected value, and since the lowest possible value is zero, and there is no limit for the highest values, the distribution is positive skewed. Poisson distributions arise under very specialized conditions, when an assumption of "pure" or simple uniformity is appropriate. However, often this assumption is not appropriate; rather there are many factors that can affect the results, all acting simultaneously so that pure or simple uniformity is not appropriate. Consequently, two parameter distributions such as a negative binomial or even binomial distribution, under certain circumstances can fit data well. However, the Poisson distribution is an important one, and in some circumstances it might provide a good fit to the data, thus the example is being given.

The C chart is used when sample size (number of units or amount of material, being sampled for one analysis) is constant for all samples; the U chart is used for circumstances where sample size may vary. Without loss of generality, it is assumed that the sample size is 1; that is, the direct counts for some material are being recorded. See Fig. 13.10 for an example of the C chart and Fig. 13.12 for an example of a U chart.

A word of caution: the Poisson distribution is a skewed distribution, thus α- and β-probabilities need to be calculated taking into consideration when the probability of being above or below the target value is not 50%. For more details on these types of charts as well as an example see Appendices 2 and 3 of AOAC (2006).

13.6.9.2 Control Chart for Binomially Distributed Data, with One Sample Size

Often qualitative analyses, for the presence of a single pathogen in samples, are performed. A chart that can be used to track the control of a process with respect to the presence of some pathogen in samples is called a "NP" – control chart. Generally such charts can be used for a binomially-like distributed characteristic (a two-class attribute test), for example, the detecting of *Salmonella* in samples. One of the classifications is assigned the name "defective" or positive, and it is that classification for which process control is measured. P refers to the percentage or probability of "defective" units (positive units of some product); the magnitude of P is to be controlled (usually to be low as possible).

The letters "NP" are used as a mnemonic for the plotting of the number of "positive results"; the expected value of the number of positive results is equal to the sample size, N, times the assumed proportion of positive samples, P – or, symbolically, NP. See Fig. 13.13a–c for an example of an NP chart. For more detailed information on how to construct this chart see Appendix 4 of AOAC (2006).

In this example, it is assumed a sample size of 50 product tests, constituting one sample, for which the number of positive results is the output. An NP-chart is a plot of the number of "positive" test results within a sample over time. The example provides methodology that can be used when the sample sizes are not the same (using a P-chart or a transformation of the results).

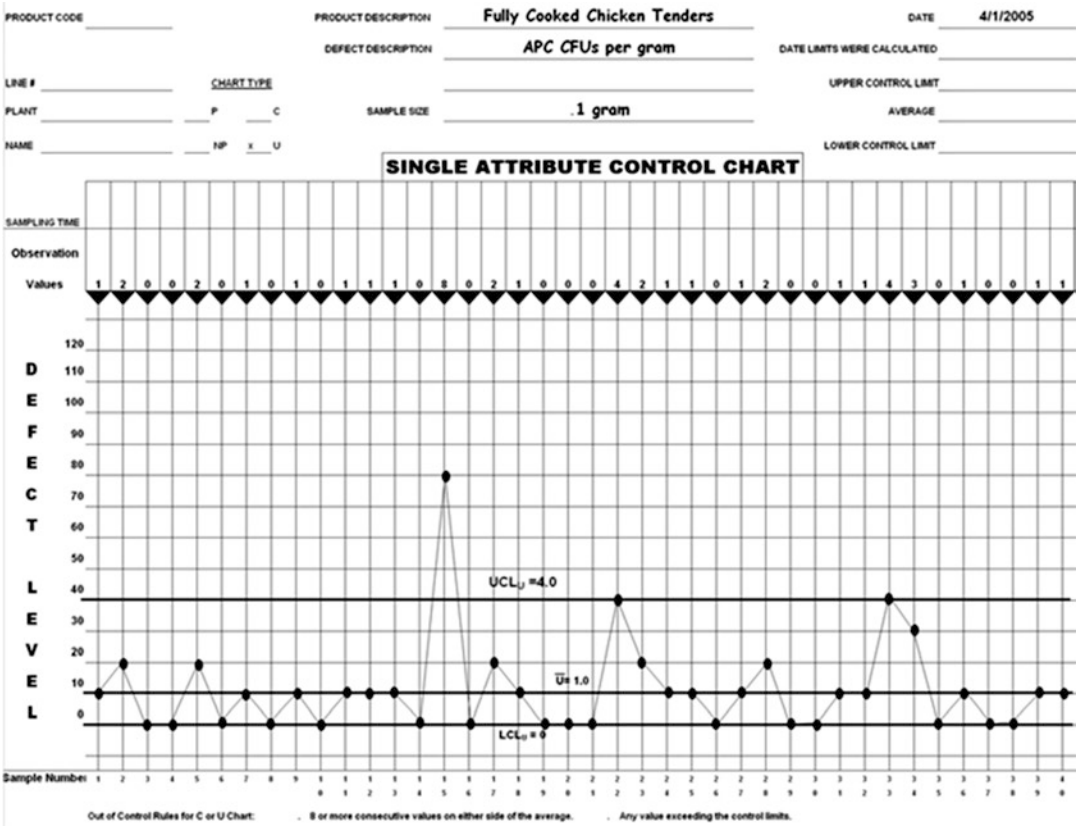

PRODUCT CODE
PRODUCT DESCRIPTION
Fully Cooked Chicken Tenders
DATE
4/1/2005
DEFECT DESCRIPTION
APC CFUs per gram
DATE LIMITS WERE CALCULATED
LINE #
CHART TYPE
UPPER CONTROL LIMIT
PLANT
P
C
SAMPLE SIZE
.1 gram
AVERAGE
NAME
NP
X
U
LOWER CONTROL LIMIT
SINGLE ATTRIBUTE CONTROL CHART
SAMPLING TIME
Observation
Values
1 2 0 0 2 0 1 0 1 0 1 1 1 0 8 0 2 1 0 0 0 4 2 1 1 0 1 2 0 0 1 1 4 3 0 1 0 0 1 1
D E F E C T
L E V E L
120 110 100 90 80 70 60 50 40 30 20 10 0
UCL_U =4.0
Ū= 1.0
LCL_U = 0
Sample Number
Out of Control Rules for C or U Chart:
. 8 or more consecutive values on either side of the average.
. Any value exceeding the control limits.

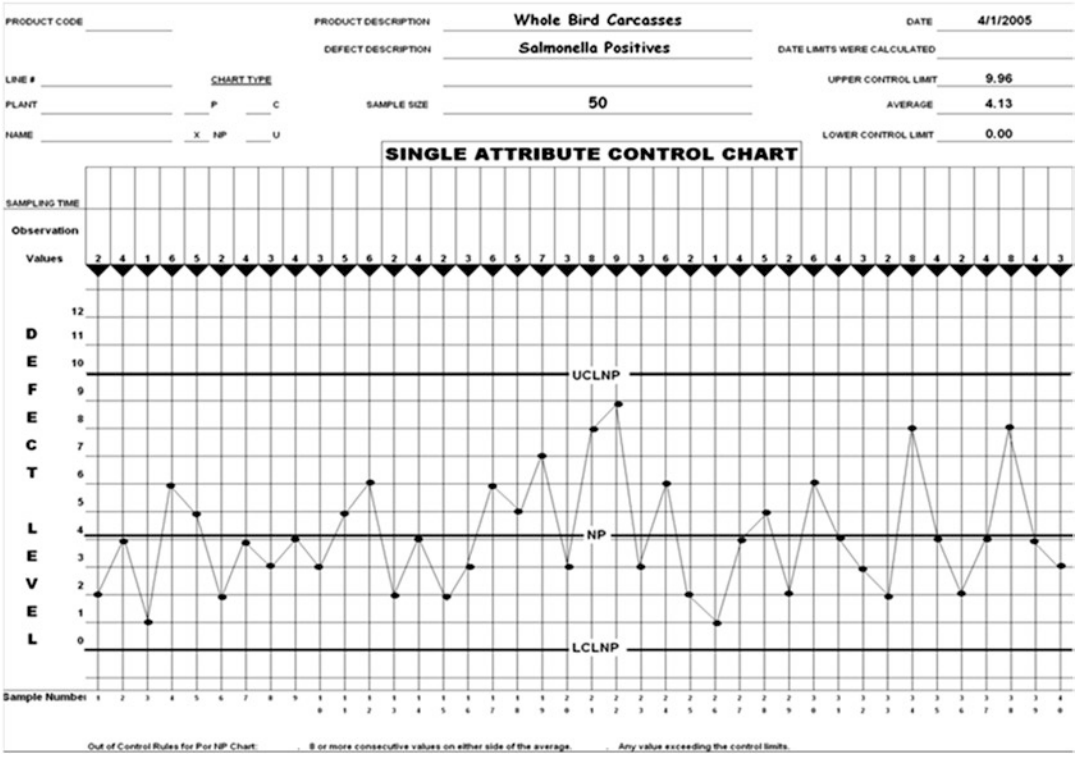
Fig. 13.12 U Chart of CFUs per gram of fully cooked product

13.6.9.3 Control Chart for Binomially Distributed Data Plotted as Proportions (P Chart), with Varying Sample Sizes

The previous example given above (refer to Sect. 13.6.9.2) of the binomial control chart plotted the number of positive results out of a sample of 50 units. The characteristic feature in that example was that the number of units per sample was fixed (= 50), so that the expected number of positive results per sample (of 50 units) was the same. However, in many situations the sample size is not the same and thus the expected number of positive results would not be the same. Thus plotting the number of positive results is not appropriate for a control chart since the underlying assumption for the data to be used for plotting, namely, that the results are from a common distribution when the process is under control, would not be satisfied.

A simple adjustment might be to plot the proportion of positive results, P_i rather than the number of positive results; however, while the expected value would be same for all samples, the expected variances of the results will no longer be the same. Thus, such data would not be usable for plotting for the reason given above. However, one possible way of correcting this is to plot: $Z_i = SQRT(N_i)\ (P_i - P)$, where P is the assumed true proportion of positive results and N_i is the sample size for the i^{th} sample. In this case, the expected value of Z is zero, and the standard deviation of Z is $[P(1\text{-}P)]^{1/2}$. For sufficiently large N_i, the distribution would be the same (approximately normal) for each plotted data point, so that the Z_i could be used for plotting a control chart. A control chart for Z would have Shewart control limits of $+3[P(1 - P)]^{1/2}$. CUSUMS and moving averages could be constructed with the Z_i values. Or, if the sample sizes were not that large, an arcsine transformation: $y_i = \sin^{-1}(P_i^{1/2})$ could be used, setting $Z_i = SQRT(N_i)\ (y_i - y)$.

If the number of distinct values of N_i is small (say two or three) it would be possible to just plot the P_i, and have two or three Shewhart limits depicted on the same chart. The following is an example of



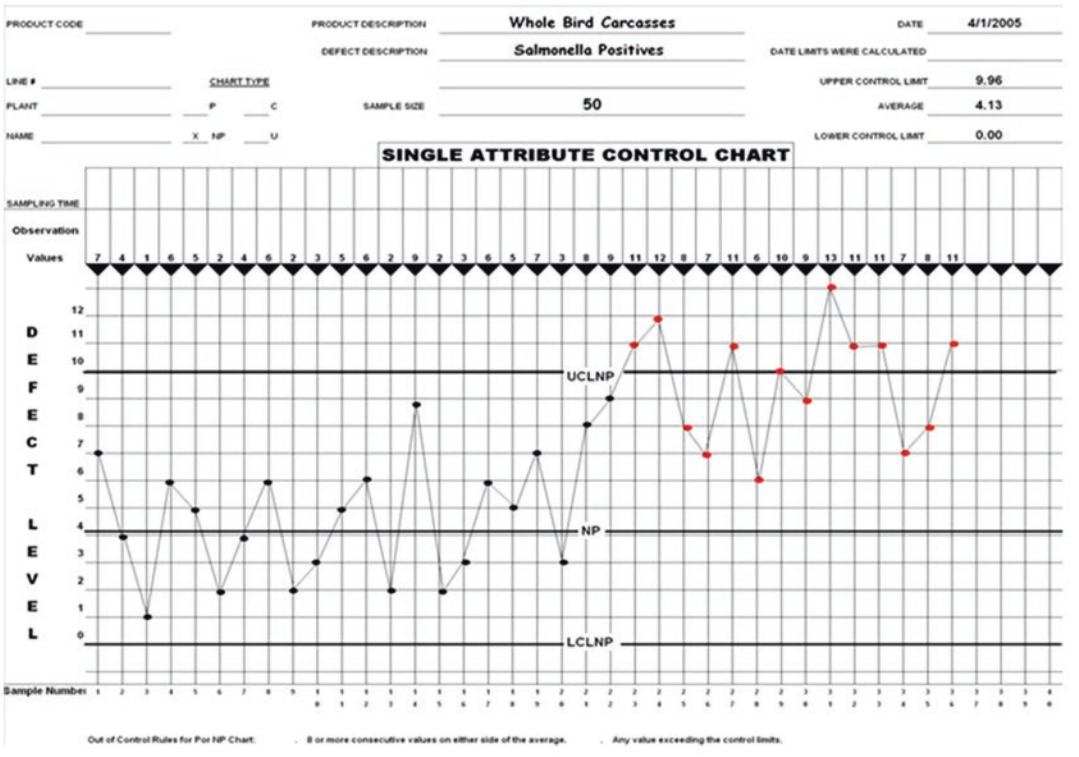


Fig. 13.13 (continued)

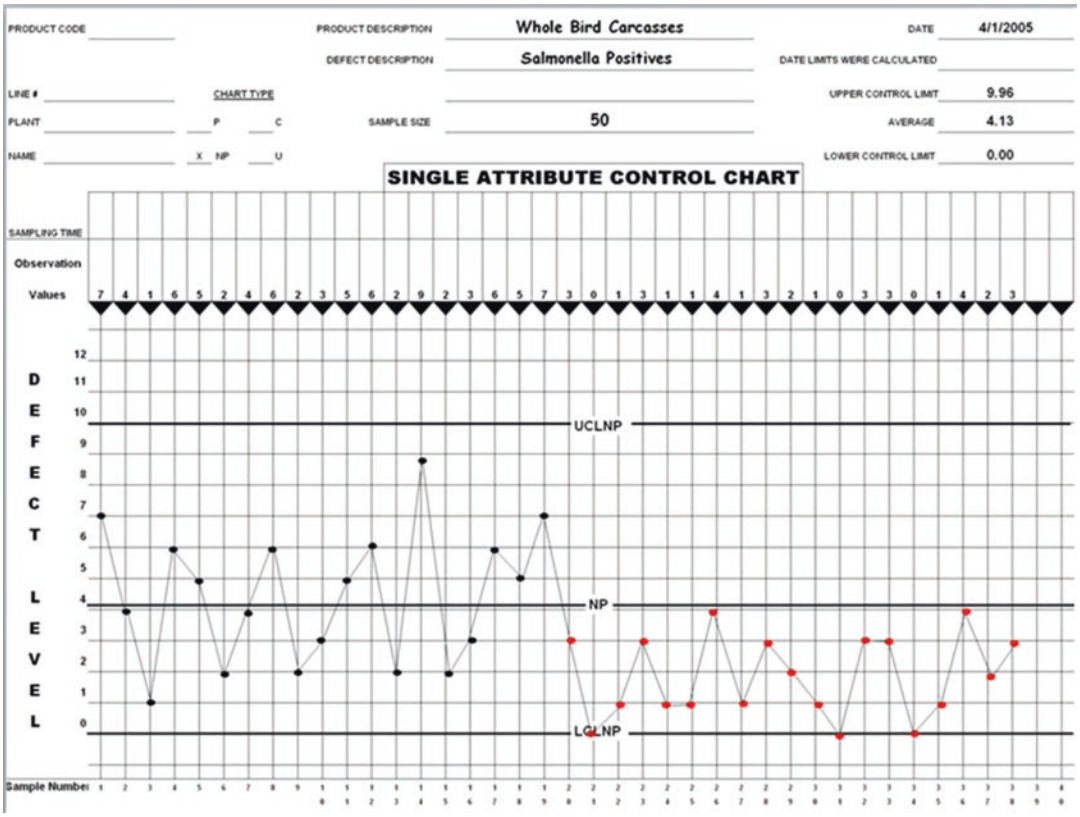


Fig. 13.13 (**a**) NP chart for base line *Salmonella* spp. data collected in sample size = 50, (**b**) NP chart showing an increase in *Salmonella* spp., (**c**) NP chart showing a reduction in *Salmonella* spp.

a P-chart with two Shewhart limits for two values of N_i (= 50 or 100). See Fig. 13.14 for an example of the P chart and Appendix 5 of AOAC (2006) for further details of this example.

13.6.9.4 Control Chart for Poisson Distribution for More than One Sample Size or When One Expresses Results in a Unit Size Not Equal to Sample Size – the U Chart

When data are collected using more than one sample size where Ni is ith sample size, and or, one expresses the results in a unit size other than the size in which the sample was collected, and an underlying Poisson –like distribution can be assumed, a U chart may be used to do process control. A U chart is a plot of observation per sample, normalized to a fixed unit size.

As an example consider the following. Aerobic plate counts (APC) are measured for a fully cooked product. Briefly, a 10% dilution is prepared: 10 g of fully cooked product is removed from post-packaged product aseptically and placed in 90 ml of diluent stomached for 60 s and then 1 ml is plated and incubated for 48 h. After the incubation CFUs are counted and data are reported as CFUs per gram. Since the actual amount of product in the 1 ml of plated diluent is actually a tenth of a gram a situation is presented where count data are reported in units other than that equal to the sample size, and counts are low. For these reasons a U chart is chosen as the chart to use for process control for this characteristic. The control chart illustrates how the user divides each observation by the sample size and plots the normalized results, (for the first observation 1 CFU is divided by 0.1which provides a value of 10, so 10 is plotted as the first point). As with the P chart, a U chart can also accommodate more than one

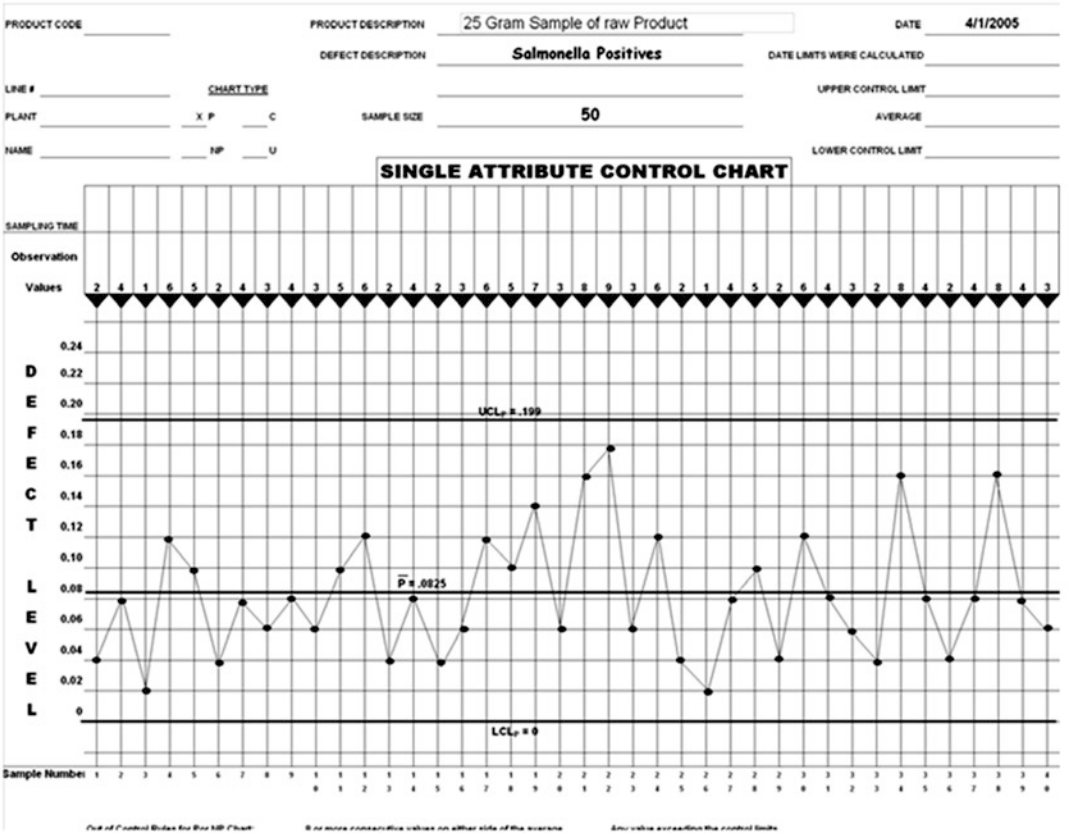


Fig. 13.14 P control chart of baseline data of *Salmonella* spp.

sample size, and, as with the P chart, the larger the sample size the closer the control limits are to the center line. (see Fig. 13.15 for a U chart example and for further details Appendix 6 of AOAC, 2006).

13.6.9.5 Control Charts for Rare Events: The Failure Control Chart, F Chart:

For events that are rare, to the extent that reasonable subgroup sample sizes would yield many zero values, a Failure Control Chart (F Chart) is an effective method for gaining an understanding as to whether the rate of the event is increasing, decreasing or remaining approximately stable. This particular chart was developed to help answer this question. The duration of time between events often follow an exponential probability distribution, which is described by a single parameter. When the process is in control, it is assumed that the value of the parameter is a specified constant.

In order to develop an F Chart, the average time between events must be estimated. After an event, the time or number of samples since the last event are determined. The distribution of the times between events is assumed to follow the exponential distribution when the process is under control. Again the rule of thumb of observing 20–30 or more events, to obtain a good estimate of the average time between events is recommended. The average time is referred to as "Mean Time Between Failure," MTBF, to keep consistency with reliability engineering convention. The probability of having not failed based on the current MTBF is calculated as follows:

$$Reliability: \mathrm{R} = Probability\ of\ not\ failing = e^{(-t/MTBF)}$$

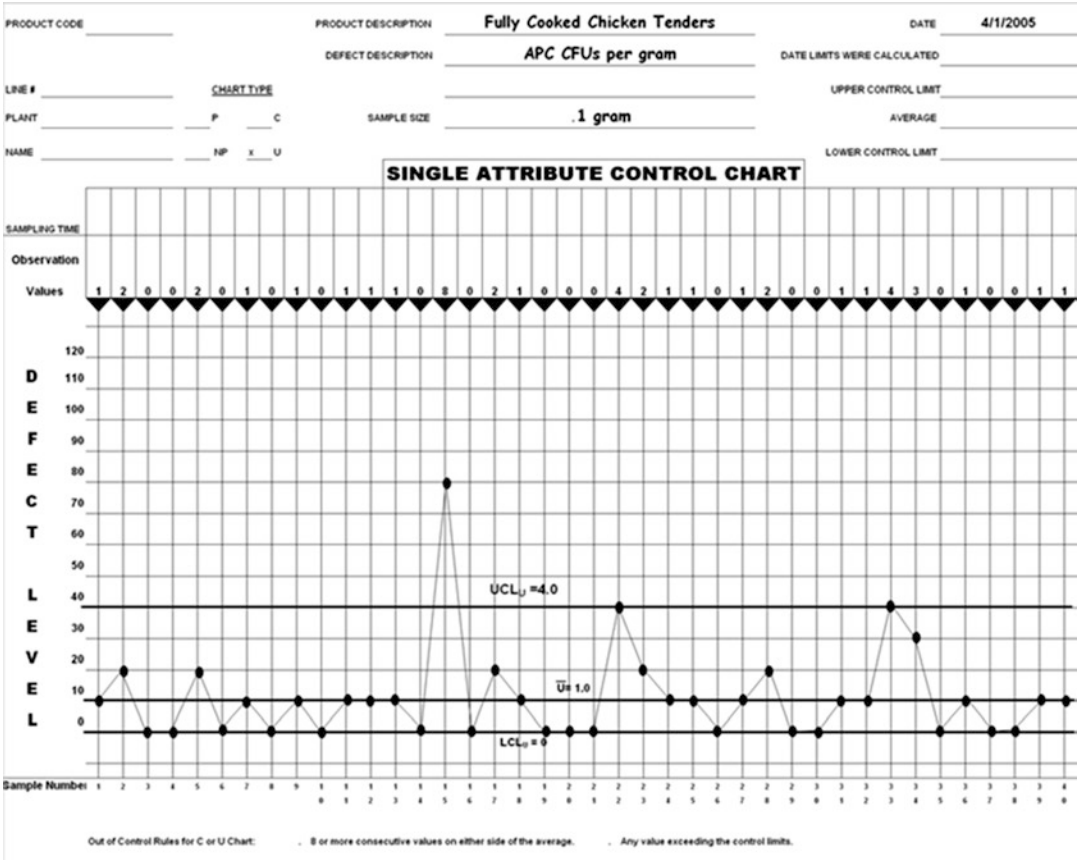


Fig. 13.15 U Chart of APC counts, CFUs per gram of fully cooked product

where t is the number of samples since the previous failure. High values of R imply low values of t, which would be undesirable.

As an illustrative example, consider the following. During a previous year, a plant collected 4400 *E. coli* 0157:H7 samples. Of those samples, 44 samples tested positive. The MTBF can be determined by dividing the number of samples by the number of positives. For this example:

$$MTBF = \frac{4400}{44} = 100$$

Time is a continuous variable and sample number is a discrete variable. This discrepancy may cause some problems when the MTBF is "small." In the example being presented, MTBF = 100, so that the probability t = 1 is 1%, since $R = e^{-1/100} = 0.99$ is the probability of not failing, so that the probability of a positive sample is 1–0.99 = 0.01. The implication of this is that two consecutive positive samples, providing an observation of t = 1, is not enough to signal "out of control" if the control limits are set where the α- probability is to be low, about 0.135%, based on the normal distribution assumption for the Shewhart (one-sided) control limit of $\mu + 3\sigma$. In order to have an α-probability that is lower, the MTBF must be no less than 750 samples.

There are many ways this "problem" can be dealt with. The easiest is to count the number of samples between positive results, exclusively, so that the above example would provide an observation of t = 0, (two consecutive events would mean no negative results between events), and thus

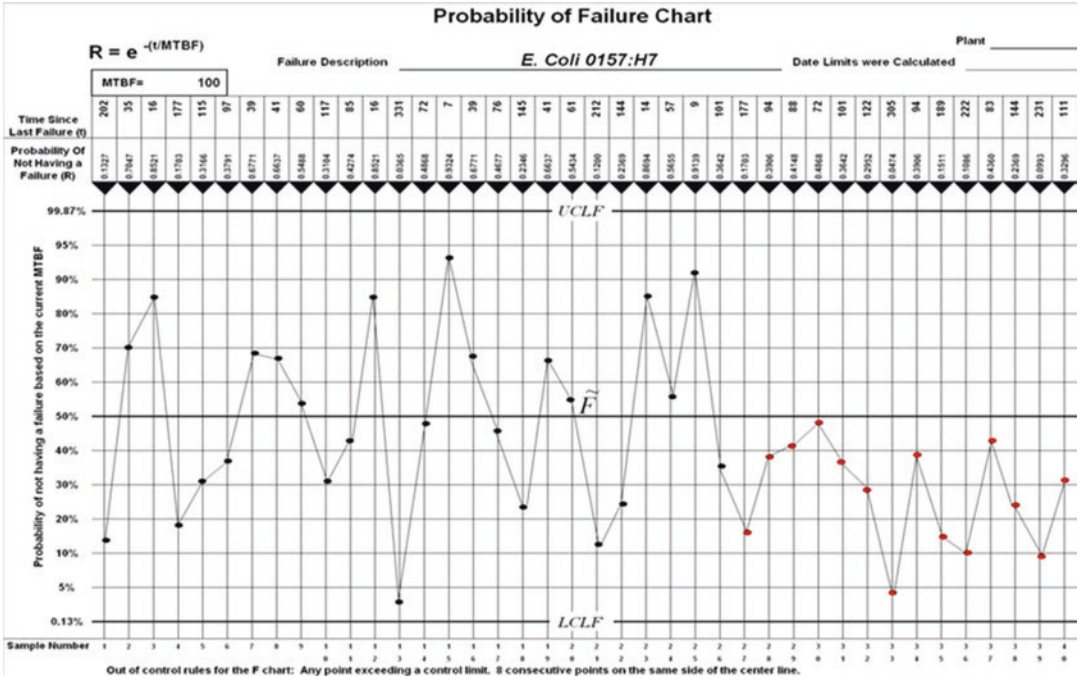


Fig. 13.16 An F Chart of *E. coli* 0157:H7 events for a process with a MTBF of 100 samples, followed by an F Chart showing an increase in the MTBF

would automatically (regardless of the value of MTBF) provide an "out of control" signal. This is a "conservative" approach insofar as it assigns the number of days the minimum it could be assuming that time was a continuous variable and what is being measured is that time when a "failure" takes place. In practice this should not create a serious bias in the α- and β-probabilities, but has the effect of increasing the α-probability slightly while decreasing the β-probability slightly over actual values. Thus, the time, t in the above formula is, (t), equal to number of samples since the last positive sample. (see Fig. 13.16 for a F chart example and for further details of the example see Appendix 7 of AOAC, 2006).

13.6.10 Advantages of Multiple Charting of Data Subsets

Verification sampling of the end product is an essential data set for effective control charting because it reflects the integration of all the process control steps and will reveal failures due to problems that affect the entire production process. However, additional verification sampling at key intermediate steps within the process can provide valuable information for isolating special causes of loss of process control. Control charting process parameters such as temperature or pH can directly contribute information to verification. Temporally aligning these variable type control charts along with attributes charts of microbiological testing data described above would enhance the overall verification program and ability of the process manager to better analyze their process. In addition, plotting subsets of a single verification data set may more rapidly identify an emerging problem. Returning to an example discussed above, suppose a plant operated three shifts per day, and they analyzed one verification sample per shift for *E. coli*. In addition to the chart of all of the test results, it would be beneficial to generate individual charts of the results for each shift. In this way, new sources of variability that were specifically associated with a single shift would be identified and isolated substantially faster.

13.6.11 Caution Regarding Control Chart Software

Control charting software is increasingly available and greatly simplifies the setting up of a variety of control charts. Caution must be observed, however, in using any of the more sophisticated options that are included in most software for advanced applications. As indicated with several examples in this chapter, food safety processing rarely satisfies all the assumptions that underlie many industrial quality control computations. Care must be taken to determine whether options selected are pertinent to the development of either attribute or variable control charts related to microorganisms or their metabolic products in foods. This is particularly important when designing control charts.

Caution should also be exercised in keeping control records only through software (and not on hardcopy). Maintaining electronic copies of original raw data sets is recommended, and is typically done through a LIMS. Some software products automatically readjust $\bar{X}$ and σ as the data are inputted. This will mask slow trends in $\bar{X}$ or σ by leaving no traces of the adjustments (Wise and Fair 1997).

13.7 Use of Process Control Testing as a Regulatory Tool

Food control agencies have traditionally relied on batch-by-batch testing for lot acceptance of foods, particularly in relation to international trade. However, with the increased emphasis on the adoption of food safety systems such as HACCP, there has been increased focus on the validation, monitoring, and verification of process control measures as the means by which food control agencies use to assess safety. An example of how these techniques can be used in a regulatory framework is the USDA Food Safety and Inspection Service's Pathogen Reduction/HACCP regulation (McNamara 1995; FSIS 1996). This regulation included two forms of microbiological testing as a means of verifying the effectiveness of HACCP programs required for the production of meat and poultry at slaughter. The first was testing of carcasses by industry for the presence of biotype I *Escherichia coli* as an indicator of fecal contamination. The second was testing for *Salmonella* spp. performed by USDA. Both were based on the application of a MSUM control charting technique, the Moving Window.

In the case of *E. coli* testing, the technique was adapted for stratified quantitative data using a limit that could not be exceeded (M-value) and a warning value (m-value) which could not be exceeded more than 3 times (c-value) in a moving window of 13 tests (n = 13). The m-value and M-value were based on national baseline surveys of the various types of meat and poultry, and are specific for that commodity (FSIS 1996). The rate of sampling (one sample taken for a set number of animals slaughtered) is also commodity specific.

The method for evaluating the carcasses for *Salmonella* was limited to presence/absence testing. Accordingly, the moving window technique was adapted for attribute testing. Again, the limits that

Table 13.3 *Salmonella* performance standards associated with the USDA Pathogen Reduction/HACCP regulation (FSIS 1996)

Class of products	Performance Standard (percent positive for *Salmonella*) (%)	Number of samples tested (n)	Maximum number of positives to achieve standard (c)
Steers/heifers	1.0	82	1
Cows/bulls	2.7	58	2
Ground beef	7.5	53	5
Broilers	20.0	51	12
Hogs	8.7	55	6
Ground Turkey	49.9	53	29
Ground chicken	44.6	53	26

indicated that the HACCP system was no longer under control were based on national baseline survey results for each commodity. The number of samples within the window and the number of positive isolations of *Salmonella* wherein the system is still considered in control varies among the different types of meat and poultry (Table 13.3).

13.8 Investigating and Learning from Previously Unrecognized Factors or Unforeseen Events

In part, HACCP verification can be viewed as additional testing conducted for the purposes of assuring that the conditions and requirements identified in the hazard analysis upon which a HACCP program was developed are still valid. As an example, consider pasteurization of liquid egg products. An obvious CCP for production of pasteurized liquid egg products is the heat step with critical time/temperature conditions applied. This CCP is effectively monitored on a real-time basis by process monitoring temperature and duration of heating. Parameters for this processing step were developed using data acquired from inoculated pack studies with *Salmonella*, and have been highly successful for controlling this pathogen in these products. However, these studies were originally conducted at a time when most eggs used to make liquid egg products were eggs left over from the marketing of shell eggs. These eggs were typically several days to weeks old. When initially laid, the white of an egg has a pH of between 7 and 8, but within several days pH rises to between 10 and 11. This elevated pH substantially decreases the heat resistance of *Salmonella*. Since development of the original heat resistance data for *Salmonella* in egg products, there has been a substantial change in the demand for liquid egg products and an increasing portion of the eggs used come directly from the egg producer. The time between laying and pasteurization has decreased to the point where the pH of egg white is still in the range of pH 7 to 8. At this pH, *Salmonella* is substantially more heat resistant. Monitoring the CCP for temperature and duration of heating alone would not detect this gradual shift in a key characteristic of the egg. However, temporally aligning the temperature/time control charts with off-line microbial data would likely enable detection of the shift. Instead, the egg industry was alerted by an increased incidence of salmonellae observed in periodic samples taken as part of effective verification programs. This is part of the verification procedure and the use of data. It is also part of change management requiring a periodic review of HACCP and validation of processing conditions. Bringing this into the SPC program may serve as a proactive indicator of change management, whereas monitoring and review of HACCP plans may be considered more reactive.

13.9 Conclusions

This chapter introduces the concept of SPC as a general method to understand the underlying system of food production. Several new concepts were presented that have analogies in the methods of food quality process control for many years. The expansion of process control to SPC is a step in the broader concept of systems thinking and approach to analyzing a food production system.

In summary, the following "performance standards" are recommended as general guidance with respect to implementing SPC systems for food safety. The performance standards are not meant to prescribe procedures or criteria that should be used for evaluating processes; rather they are meant to provide guidance and a methodology to be used for developing a SPC sampling plan. These principles are adapted from Appendix F, Enclosure B, AOAC (2006) are offered as guidance and for reference:

Performance standard 1. Charts or plots of the output data over time are not only valuable for verifying calculations and having a visual picture of the variation exhibited by the process output, but

also it is an integral tool to be used for identifying sources of unexpected variation in output leading to their elimination. Thus charting is a necessary tool to gain the full benefit of doing SPC.

Performance standard 2. Results to be plotted in a control chart, when the process is under control, used for SPC should be Normal or nearly Normally distributed. In cases where this is not true and an alternative known distribution cannot be assumed such as a Poisson, binomial, or negative binomial distributions, transformations such as the log transformation, arcsine transformations for binomial data, or a square root transformation for data distributed nearly as a Poisson distribution should be considered.

Performance standard 3. During some "initial" period of time, when the process is operating in a relatively stable manner, i.e., is in control, a process control study should be conducted. During this period the distribution of the measurements should be estimated and rules for evaluating the process should be formulated (the OCAP). The statistical "rule of thumb" of using about 20–30 results (or more) for computing means and standard deviations or other summary statistics needed to estimate the distribution of results and construct control limits is a recommended and desirable goal.

Performance standard 4. Rules for evaluating process control should be set considering the two types of errors: Type I, declaring the process out of control when it is not, and Type II, not declaring a process out of control when it is. Typically there are two measures, depending upon the nature of the rule, that are used for assessing these errors: (1) the probabilities of the two types of errors at a given time (referred to as α- and β- probabilities, respectively); and (2) the average run length (ARL) – the expected number of samples before an out of control signal (one of the rules being not met) is seen.

Performance standard 5. When a process is thought to be "in control," the limits for assessing individual results are set at some distance from the average, expressed as standard deviation units from the mean or process target value. The default distance is 3 standard deviations. Limits other than these should be implemented when taking into consideration economic and public safety of incorrect decisions regarding whether the process is in control. When developing rules, the α-probability (for the Type I error) should be kept low, for example, below 1%.

Performance standard 6. There are numerous run/trend rules that can be used, such as runs test, moving averages and CUSUMS, for detecting shifts in the process mean; and rules for detecting shifts in the process variation or other auto-correlated patterns that could be due to systematic source of variation. The use of any of these may depend upon particular expected conditions that arise when the process is out of control, and the sensitivity desired for detecting such conditions. In assessing the use of these rules, consideration of the average run length (ARL) is a recommended when the process is in control, ARLs exceeding 100 are typical (corresponding to a less than a 1% α – error).

Performance standard 7. "Specification Limits" are not SPC limits; specifications are either customer, engineering, or regulatory imposed/related. Statistical process control limits are driven by the process mean and its variability. Specification limits should not be placed on a control chart insofar as these might be considered as process goals thus influencing the efficacy of SPC procedures for ensuring a controlled process, and thereby undermining the safety of the product.

The SPC charting system can be important aid in identifying when and where an investigation for a cause for the process being out of control should commence. A low α-probability does not imply that, when a process is in control, "out of control" signals would not occur**.** However, since these occurrences are not expected frequently, the occurrence of one encourages an examination of the process in search for "assignable causes" for each out of control signal. However, if out of control signals occur more frequently than what would be implied by the α-probability, random chance –the unlucky draw- should be ruled out as a possible reason for the signals, and that there is an "assignable cause" for the excessive variation in the process output and/or one or more of the process parameters are incorrectly set. This would then call for a more rigorous review or further study of the process such as a root cause

analysis, (Montgomery 2009). If the plot of the data shows an abrupt change from consistently being in control to consistently being out of control, then it can be concluded with high confidence that there has been an enduring failure somewhere in the process that requires immediate remediation. The plotting of the process may reveal a gradual, progress loss of control over a series of lots. This pattern could result, for example, from a piece of equipment steadily becoming out of adjustment or a progressive environmental contamination resulting from an inadequate sanitation program. Another pattern could show a transitory but reoccurring or cyclical loss of control, e.g., every Monday morning. While no explicit criteria are given for detecting these types of cyclical patterns, one could use the "run rules" of 8 in a row, e.g., if for 8 Mondays, the plotted point is above the target value, it would be suggested that for some reason results for Monday are "out of control." The SPC plots can also document improvement in process control resulting from deliberate alterations or added mitigations. The lower levels due to the process changes are used to establish new process standards.

When the limits for declaring a process out of control are exceeded too frequently, a producer always has the option to accept the implied non-desirable or optimal processing. Whether this option is taken depends upon 'costs' (technical feasibility, monetary, quality, nutritional value) of fixing the problem, e.g., taking measures that would reduce either the process mean or the process variation. For example, a process mean level of some microbiological indicator variable could be reduced by increasing the heat processing temperature. However, this mitigation requires more energy consumption and may reduce sensory and nutritional quality of the food product. Reducing the variation might be accomplished by simply improving the air circulation within the oven or the one-time expense of a new oven. This mitigation would likely have an additional benefit of reducing the proportion of product that was over cooked, thereby improving the sensory and nutritional quality. This simple example shows a general rule: **it is generally more advantageous to reduce variation first**. If that is not successful, then a process step(s) may need to be redesigned to lower the entire distribution by lowering the process mean. However, when the limits are exceeded at a frequency that exceeds what would be expected by chance alone, it is an indication that one or more process parameters have changed significantly or a critical control point was missed; in this situation a review and revalidation the process HACCP / GHP plan(s) is warranted.

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Chapter 14
Aflatoxins in Peanuts

14.1 Introduction

Aflatoxins are chemical metabolites produced mainly by *Aspergillus* species in food held under favorable conditions. They are the most potent liver carcinogens known, capable of causing cancer in all animal species studied including humans.

The primary *Aspergillus* spp. that are sources of aflatoxins are *Aspergillus flavus, A. parasiticus* and *A. nomius*. Other less common *Aspergillus* species that may also produce aflatoxins are *A. arachidicola*, *A. bombycis, A. minisclerotigenes, A. parvisclerotigenes*, *A. pseudocaelatus*, *A. pseudonomius*, *A. pseudotamarii*, *A. togoensis, A. mottae, A. sergii, A. transmontanensis* and *A. novoparasiticus* (Pildain et al. 2008; Varga et al. 2011; Soares et al. 2012; Gonçalves et al. 2012; Massi et al. 2014). *Aspergillus flavus* is found in tropical and warm temperate zones and is associated with peanuts, maize and cottonseed. It also occurs in tree nuts, especially pistachios and Brazil nuts. Evidence suggests that *A. parasiticus* has a more limited geographical range than *A. flavus* being predominant in peanuts. *A. nomius* has been considered as a major source of aflatoxins in Brazil nuts, but is prone to misidentification, which may underestimate its presence (Pitt and Hocking 2009; Olsen et al. 2008; Calderari et al. 2013).

The four major naturally produced aflatoxins are known as B_1, B_2, G_1 and G_2. 'B' and 'G' refer to the blue and green fluorescent colors produced under UV light on thin layer chromatography plates, while the subscript numbers 1 and 2 indicate elution order on the plates. *A. flavus* produces only B aflatoxins, and only about 40–60% of isolates are toxin producers, though percentages of toxin producing isolates may vary. *A. parasiticus* and *A. nomius* produce both B and G aflatoxins and most of the isolates are producers (Frisvad et al. 2006; Pitt and Hocking 2009; Taniwaki and Pitt 2013).

Aflatoxins are extremely toxic, and the worldwide regulatory levels for aflatoxins (B_1, B_2, G_1 and G_2) range from 1 to 35 μg/kg (FAO 2004). Most regulatory limits are based on the concentration of aflatoxin B_1. Analyses of aflatoxins in foods are usually performed using high performance liquid chromatography. The traditional thin layer chromatography method is inexpensive and reliable and is still being used in less developed economies. For acceptable/not acceptable testing, immunochemical methods are most frequently used. For advanced users such as high volume analytical laboratories or regulatory authorities, liquid chromatography coupled with mass spectroscopy has become normal practice, the limits of detection are below 0.1 μg/kg (Krska et al. 2008; Shephard et al. 2010).

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Peanuts, which develop underground, become vulnerable to fungal invasion and insect attack before harvest. Several species of fungi are found in peanuts, but the presence of *A. flavus* and the production of aflatoxins is the major public health concern. Aflatoxins in peanuts are very unevenly distributed so, as a consequence, aflatoxin levels must be based on representative samples obtained using carefully constructed sampling plans. Samples must be ground or finely divided then subsampled for assay. Assays based on small samples are unreliable. In more homogeneous products, e.g. peanut butter, this is less of a problem (ICMSF 2002).

14.2 Risk Evaluation

14.2.1 Risk Assessment

Unlike infectious microbiological hazards, aflatoxins are chemical contaminants produced by fungi in crops before or after harvest. Their levels are unpredictable and can vary both temporally between seasons and spatially between different growing areas or under different storage conditions. As the elimination of mycotoxins is, generally, not possible, risk assessments are undertaken to guide food regulators and scientists in undertaking risk management processes, such as the setting of legislative levels or guideline targets for mycotoxins in food supplies (Shephard 2008). Therefore, risk assessment as a process of quantifying the magnitude of exposure and the subsequent probability of a harmful effect to individuals or populations is an important tool in evaluating potential health implications of mycotoxin exposure.

Risk assessment including hazard identification, hazard characterization, exposure assessment and risk characterization, has been performed for aflatoxins by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA). Although the hazard identification and characterization are inherent functions of the toxin, the exposure assessments and, consequently, the risk characterization are clearly influenced by the type and quantities of food consumed by individual populations or groups within a population (WHO 1998).

Aflatoxins have long been known to produce five distinct effects: acute toxicity, liver carcinogenicity, liver cirrhosis, immune-suppression, and growth retardation in children. Aflatoxins are among the most potent mutagenic and carcinogenic substances known, both acutely and chronically toxic to animals and humans. The International Agency for Research on Cancer (IARC 1993) recognizes aflatoxin B_1 and naturally occurring mixtures of aflatoxins as Class 1 carcinogens, i.e. they are recognized as carcinogenic to humans. In the liver, aflatoxin B_1 is converted by cytochrome P450 enzymes to the 8–9 epoxide. This substance is capable of binding to liver proteins, leading to liver failure and potentially to aflatoxicosis. This epoxide is also able to bind to DNA, a precursor step to the development of liver cancer. Aflatoxin B_1 is consistently genotoxic in vitro and in vivo (IARC 1993, 2002).

Extensive experimental evidence has shown that aflatoxins are capable of inducing liver cancer in many animal species. However, translating that information to humans has proved to be extremely difficult (ICMSF 2002). Evidence has been presented indicating metabolic activation of aflatoxin B_1 in fetal liver (Wild et al. 1991). The presence of aflatoxins in breast milk and weaning food in certain African countries indicates that exposure to humans can begin at the earliest age and continue throughout life (Shephard 2008). Exposure to high levels of aflatoxins can result in acute human aflatoxicosis leading to jaundice, edema, hemorrhage and death (Shephard 2008).

JECFA (WHO 1998) has reviewed dose response analyses performed on aflatoxins. All of these analyses suffer limitations, of which three are especially important. First, all of the epidemiological data from which a dose response relationship can be developed are confounded by concurrent hepatitis B infection. The epidemiological data are from geographic areas where both the prevalence of hepatitis B positive individuals and aflatoxins is high; the relationship between these risk factors in

areas of low aflatoxin contamination and low hepatitis B prevalence is unknown. Second, the reliability and precision of the estimates of aflatoxin exposure in the relevant study populations are unknown. In particular, the biological markers currently used to indicate aflatoxin intake by humans do not reflect long term aflatoxin intake, and in most cases analyses of crops for aflatoxins do not take account of reduction in levels of aflatoxins consumed in foods after processing. Third, the shape of the dose response relationship is unknown, which introduces an additional element of uncertainty when choosing mathematical models for interpolation (ICMSF 2002).

Approaches to the characterization of genotoxic and carcinogenic compounds (non-threshold response) include the mathematical modeling of the dose response curve to estimate a low effect level (benchmark dose) or the carcinogenic potency (WHO 1998). Observations concerning the interaction of hepatitis B and aflatoxins suggest that two separate aflatoxin potencies exist, one in populations in which chronic hepatitis infections are common and the second where such infections are rare. As a consequence, JECFA divided potency estimates for analyses based on toxicological and epidemiological data into two basic groups, applicable to individuals with and without hepatitis B infection. Despite differences in mathematical models, JECFA found these estimates useful as they mean that a broad range of possible values is covered. Epidemiological data for which potencies were calculated without knowledge of hepatitis B status were also reviewed and found to be in the range of potencies for hepatitis B infected or uninfected individuals. JECFA also looked at the extrapolation of animal data to estimate potency in humans; these also generally fell within the range of the potency estimates derived from the epidemiological data (WHO 1998; ICMSF 2002). In contrast to this high level of cancer risk in developing countries, much lower risks are associated with aflatoxins in developed countries due to their lower hepatitis prevalence rates and lower exposure as a consequence of better food quality and lower consumption of potential aflatoxin contaminated foodstuffs (Shephard 2008).

14.2.2 Exposure Assessment

Exposure assessment is variable across populations and subgroups of populations. It is dependent on the levels of contamination present and on the quantities of contaminated food consumed by individuals. In the case of peanuts, exposure is highest in regions such as sub-Saharan Africa and South-East Asia where peanuts are consumed as a staple food.

Depending on the objectives of the assessment and the availability of data, two methods can be used to estimate the exposure. In the deterministic model, or point estimate, fixed values of concentration and consumption *per* body weight are used to calculate intake, such as the mean or a given percentile. In the probabilistic model, concentration and consumption variables are described as distributions, and statistically modeled using techniques such as Monte Carlo stimulations (Kroes et al. 2002; van Klaveren and Boon 2009). The combination of stochastic modeling and data on food contamination levels and consumption provides a tool in determining the distribution of toxin exposure in different communities.

Without accurate exposure data, quantitative risk assessment can be limited, because exposure is a major component of the calculations. Biomarkers for aflatoxin exposure, internal dose, and biologically effective dose are increasingly being used to estimate human exposure. Measurement of biomarkers related to aflatoxin exposure (e.g., DNA adducts) typically requires samples of either urine or serum (Groopman et al. 2008; Wild and Gong 2010). JECFA assumed a population with a European diet, from which all samples containing over 20 μg/kg aflatoxin had been removed. The mean aflatoxin intake for this population was 19 ng per person per day. Assuming a 60 kg person, the mean cancer risk for that population was 0.004 cancers per 100,000 population per annum (WHO 1998). A study in China suggests a level of exposure to aflatoxin in peanuts of 0.218–0.222 ng/kg BW/day for children; while that in adults was 0.106–0.108 ng/kg BW/day (Ding et al. 2012). In African countries,

population risk based on exposure to aflatoxins is substantially higher. In a number of cases, the levels are of sufficient concern for risk managers to consider action among population groups most liable to exposure. In Gambia, several stable foods including maize, millet, sorghum, rice and groundnuts, are contaminated with aflatoxins and the total exposure becomes the sum of the exposure from individual sources. Groundnut consumption in Gambia is high and the mean aflatoxin intake is calculated at 16 ng per person per day. The population cancer risk per year in Gambia as a developing country was obtained as the product of the exposure data and an average potency figure arrived at from the individual potencies of HBsAg+ and HBsAg- groups in which the HBsAg+ prevalence rate was assumed to be 25%, resulting in 1.3 cancers per 100,000 population annum (WHO 1998). The dietary exposure to aflatoxins in European Union ranged from 0.93 to 2.45 ng/kg BW/day for lower bound to upper bound (Leblanc et al. 2005; EFSA 2007). In the United States, exposure was estimated at 2.7 ng/kg BW/day (JECFA 2008) and in Japan an average of 0.73 ng/kg BW/day (Sugita-Konishi et al. 2010).

Actual liver cancer incidence rates in males per 100,000, vary widely across different countries, with high rates in Middle, Western and Southern Africa (18.9–13.9), sub-Saharian Africa (13.1) and South Eastern Asia (21.4) to low rates in North America (6.8), Western and Northern Europe (7.2–4.1) and Latin America and Caribbean (5.8) (IARC 2008). These data do not correlate directly with the consumption of food contaminated with aflatoxins, since there are other factors causing liver cancer in humans. However, the difference in regional exposures to aflatoxin is consistent with higher incidences of liver cancer.

14.2.3 Factors Affecting Exposure Assessment

Some epidemiological studies have suggested that intake of aflatoxins alone poses a detectable risk but others studies indicated that the presence of other factors, such as hepatitis B virus (HBV), are necessary to induce liver cancer. The identification of hepatitis C virus (HCV) is an important advance in understanding the etiology of liver cancer. Epidemiological studies are largely consistent in showing a strong association between antigens to HCV and the occurrence of liver cancer. The risk linked to HCV is independent of HBV and other risk factors. HCV is likely to be the major cause of liver cancer in countries with low to medium risk of liver cancer, such as the United States, Europe and Australia (ICMSF 2002). The epidemiological evidence of the carcinogenicity of HCV has been reviewed and endorsed by an international group under the leadership of the IARC (1993).

Viral hepatitis is a major worldwide public health problem. It is estimated that over 300 million individuals are chronically infected with hepatitis B (HBV) and perhaps 100 million with hepatitis C (HCV). Although the evidence remains inconclusive, it is estimated that 50–100% of liver cancer cases worldwide are associated with persistent infection with hepatitis B and/or hepatitis C. HBV is prevalent in the developing parts of the world, and HCV is emerging as a major cause of hepatocellular cancer in Japan and western societies (Bosch 1997). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) estimated the carcinogenity potency of aflatoxins for individuals with the hepatitis B virus (HBsAg+) to be 0.3 cancers/year/100,000 individuals (P HBsAg+), 30 times higher than the potency for non-infected individuals (0.01 cancer/year/100,000 individuals; P $HBsAg^{-}$) (WHO 1998).

More recently, a review of re-analysis of overall evidence from epidemiological studies (Pitt et al. 2012) showed that a particularly elevated risk of hepatocellular carcinoma (HCC) from aflatoxin exposure in individuals chronically infected with HBV and reasonable evidence that an increased risk also exists in individuals exposed to aflatoxins without chronic HBV infection. Given that more than 350 million chronic HBV carriers exist worldwide, many living in aflatoxin-endemic areas, the need to reduce aflatoxin exposure remains highly relevant for cancer prevention (Pitt et al. 2012).

14.2.4 Risk Characterization

JECFA (2008) reviewed the potencies of aflatoxins estimated from the positive epidemiological studies and chose separate central tendency estimated potencies and ranges for hepatitis B positive and hepatitis B negative individuals. Potency values chosen for positive individuals were 0.3 cancers per year per 100,000 population per ng aflatoxin ingested per kg body weight per day with an uncertainty range of 0.05–0.5. For negative individuals there were 0.01 cancers per year per 100,000 population per ng aflatoxin per kg body weight per day with an uncertainty range of 0.002–0.03. Using the above assumption, a study suggests that the risk of liver cancer due to peanut consumption in China, for example, is 0.003–0.17 cancer cases/100,000/year (Ding et al. 2012). Assuming a 60 kg person, the mean cancer risk for this population was 0.004 cancers per 100,000 population per annum.

Liu and Wu (2010) estimated the global burden of hepatocellular carcinoma (HCC) induced by aflatoxin by using the quantitative cancer risk assessment and collecting national data on foodborne aflatoxin levels, consumption levels of maize and peanuts and HBV prevalence. The cancer potencies of aflatoxin for HBV-positive and HBV-negative individuals were considered, together with uncertainties in all variables. Liu and Wu (2010) estimated that of the 550,000–600,000 new HCC cases worldwide per year, about 25,200–155,000 may be attributed to aflatoxin exposure. In other words, aflatoxin may play a causative role in 4.6–28.2% of all global cases. Most cases occur in sub-Saharan Africa, South-East Asia, and China, where populations suffer from both high HBV prevalence and largely uncontrolled exposure to aflatoxin in food.

14.3 Risk Management

Aflatoxin risk in peanuts can be managed either by governmental regulations that specify a maximum tolerable limit of aflatoxins in peanuts or by agricultural and public health interventions that can be adopted to reduce levels of aflatoxins in peanuts.

Because aflatoxins are a well-known chemical hazard (albeit from a microbial source), risk management has taken a different path from that expected for bacteria or bacterial toxins. In the years following the discovery of aflatoxins, the limits set for aflatoxins in foods reflected the limits of detection of the chemical assays. In importing countries, this was initially set at 5 μg/kg, then in some cases reduced to as low as 1 μg/kg (van Egmond 1989). However, it soon became clear that producing countries could not meet such limits: the United States set 25 μg/kg and Australia 15 μg/kg as practical limits which would reduce aflatoxin ingestion as far as possible without damaging the peanut industries in those countries (ICMSF 2002). In 1999 the Codex Alimentarius Committee on Food Additives and Contaminants (CCFAC) adopted a maximum level of 15 μg/kg for total aflatoxins in peanuts for further processing, including a standardized sampling plan (CAC 2001). For ready-to-eat peanuts the Codex Committee on Contaminants in Foods (CCCF) is still discussing the establishment of a maximum level of aflatoxins (CAC 2013).

Epidemiologic and animal studies established that aflatoxins are genotoxic carcinogens, lacking a NOAEL (apparent No Observed Adverse Effect Level). Equations for cancer incidence in relation to aflatoxin consumption were developed, but due to a lack of epidemiological evidence, were not universally accepted because of the probable interaction with hepatitis B and C viruses. As a consequence, limits continued to be set more on the basis of perceived risk in importing countries, or on attainable levels in developed exporting countries. In developed countries aflatoxin regulations have reduced foodborne risk because enforcement is strong and technologies and methods exist to reduce or remove the contamination. However, the risk of aflatoxin contamination in peanuts is greatest in developing countries, where agricultural systems are often poorly equipped to handle food safety

risks. Sub-optimal field practices and poor storage conditions make the crops vulnerable to fungal infection and subsequent aflatoxin accumulation. The problem is exacerbated by the fact that peanuts are a staple food in the diets of many people worldwide, and thus aflatoxin exposure is higher where dietary variety is difficult to achieve (Shephard 2008). Hence, regulatory standards for mycotoxins in food sometimes have no impact, or even potentially adverse impacts, on human health in developing countries (Wu 2004; Shephard 2008). Instead, the focus for risk management should be on technologies and public health interventions to reduce mycotoxin risk, infrastructures to support these technologies and public education (Pitt et al. 2012).

14.3.1 Principal Control Measures

Controlling aflatoxins in peanuts is not easy. Under the drought stress conditions that often prevail when peanuts are grown as a dry culture crop, aflatoxins may be produced before the nuts are pulled from the ground. Under these conditions, it is clear that control of aflatoxin formation by measures taken after that point cannot be totally effective. Aflatoxins are also quite resistant to normal food processing, including heating (ICMSF 1996), so processes used to reduce bacterial or fungal contamination cannot be relied on to remove aflatoxins. However, aflatoxins in peanuts can be managed at various points along the food production chain from farm to fork. Good Agricultural Practice (GAP), Good Hygiene Practices (GHP), Good Manufacturing practice (GMP) and Hazard Analysis and Critical Control Point (HACCP) principles are the primary tools to achieve Food Safety Objectives (FSO), thus FSO should be based on a realistic assessment of what can be achieved through these (ICMSF 2002; Pitt et al. 2013).

14.3.1.1 Primary Production

Initial Level of Contamination (H_o)

A variety of factors influence invasion of *A. flavus* and *A. parasiticus* in peanuts. Infection before harvest occurs only if substantial numbers of fungal conidia exist in the soil. In a bad season under drought stress conditions which often prevail when peanuts are grown as a dry culture crop, aflatoxins may be produced before the nuts are pulled from the ground (Pitt et al. 2012). The major factors influencing *A. flavus* and *A. parasiticus* infection in peanuts are insect damage to the developing nuts and plant stress due to drought and high soil temperatures before harvest. Drought stress acts in three ways: first, by wilting the plant and reducing its metabolic activity, which decrease the plant's natural defenses against fungal infection; second, by reducing the water activity in the soil, which reduces growth and activity of competing microorganisms; and third, by promoting growth of *A. flavus* and *A. parasiticus*, which are xerotolerant (Pitt and Hocking 2009). Reducing drought stress by irrigation or rain; limiting insect damage by GAP; reducing spore numbers by crop rotation; or introducing non-toxigenic strains of *A. flavus* for competitive exclusion (Dorner and Horn 2007; Pitt et al. 2012), all assist in reducing the occurrence of aflatoxins before harvest. However, drought stress cannot be prevented under the dry culture condition, which is the most widely used to produce peanuts.

Aflatoxins are also formed while peanuts are being dried, which is usually carried out in the field, and can also continue during farm storage if drying has been inadequate or when storage is carried out at high relative humidity (RH) and/or temperature. Good agricultural practice can assist in limiting aflatoxin formation, but not completely prevent its production (Pitt et al. 2012).

Increase During Storage ($\sum$I)

Provided peanuts are adequately dried on the farm and maintained in a dry state during transport and storage, the fungi which produce aflatoxins cannot grow, thus no increase in aflatoxins should occur ($\sum I = 0$). FAO (2001) recommended that the dried peanut reach moisture content of 5–6% and storage at 10 °C or below 65–70% RH. Generally, a RH of 85% or less inhibits the growth of *A. flavus* in peanuts (Hilmy et al. 1995). However, in some tropical countries, where high humidity conditions prevail and storage conditions are inadequate, aflatoxigenic fungi can grow and aflatoxins can continue to be formed. Control of moisture content and/or temperature of storage to prevent aflatoxin production has been reported for pistachio nuts, i.e. storage at 10% moisture content at any temperature or at any moisture content at temperature ≤10 °C (Garcia-Cela et al. 2012).

14.3.1.2 Manufacturing: Reduction in Levels During Processing ($\sum$R)

Several procedures, such as color sorting, density flotation, blanching and roasting, can be used by processors to reduce aflatoxin levels by as much as 99% (Park 1993; López-García et al. 1999). Aflatoxins in peanuts can be decreased at any stage of production by removing defective nuts and also other foreign matter by hand sorting, winnowing, and separation by gravity or other methods. Sorting moldy nuts or kernels by hand and eye has proven a particularly effective method of removing a large proportion of aflatoxin contamination in the food (Turner et al. 2005).

The major method used in industry is color sorting. In this procedure nuts are inspected individually by an electronic or laser sorting system and discolored nuts are removed. The rationale for aflatoxin reduction by color sorting is that the growth of a fungus in a peanut results in discoloration, so removal of discolored nuts sorts out those containing aflatoxins as well. In the United States and Australia it is a standard commercial practice that every individual shelled peanut entering commerce has been color sorted. If the color sorting process is ineffective, as can occur when severe drought stress causes peanuts to commence drying in the soil before harvest, it is common practice to blanch peanuts to remove their skins, then roast and color sort them again. This accentuates the darkening process and facilitates color sorting (Pitt et al. 2012).

Blanching is a process which involves the roasting of raw peanuts with the aim of loosening skins for easy removal by blanching rollers. The nut coat is removed and then the blanched nuts are sorted for discoloration using electronic color sorters. Electronic color sorting after removing the nut coat is believed to be more efficient for removing the damaged nuts (which may contain aflatoxins) than color sorting nuts prior blanching, because of the increased contrast between the damaged and a white nut background. Those peanuts that have a different color are removed from the process by a jet of compressed air. Therefore, damaged peanuts will be detected by the color sorters and removed from peanut stream. This action showed reduction of aflatoxin levels in peanuts of 87–91% (Whitaker 1997).

It is normal practice to assay aflatoxin levels in all consignments of peanuts in major developed producing countries, often repeatedly, from shellers to final product. The aflatoxin level is checked by careful sampling, preferably on line, and analyses to meet the performance objective (PO), and eventually the FSO value (ICMSF 2002). Such controls rarely exist in less developed countries. Other measures applied to reduce aflatoxins also include boiling (Njapau et al. 1998), extrusion (Cazzaniga et al. 2001) and autoclaving (Stoloff et al. 1978). Dry roasting of peanuts can reduce aflatoxin levels by up to 80% (Njapau et al. 1998). Heating of peanut oil at 250 °C for 3.5 h reduced aflatoxin by 99% (Peers and Linsell 1975). The alkali process used to produce refined table oil completely removes aflatoxins (ICMSF 1996).

14.3.1.3 Distribution/Marketing

Distribution of processed peanuts should be carried out at the appropriate RH and temperature. For post processing storage RH below 70% and temperature between 0 and 10 °C is recommended depending on expected storage duration (FAO 2001). The lower the temperature, the longer the storage life.

14.3.1.4 Preparation/Consumption

Roasted peanuts are generally a ready to eat product and no further increase or decrease in aflatoxins is expected to occur especially when moisture content of the product is maintained at low level.

14.3.2 Establishing ALOP/FSO

The development of an Acceptable Level of Protection (ALOP) for aflatoxins has proved very difficult because a no-effect level has not been established. It is assumed that there is no threshold of exposure to aflatoxin B_1 below which cancer would never occur, because aflatoxin B_1 has a reactive metabolite that interacts directly with DNA (Pitt et al. 2012). On one hand it could be argued that an ALOP should be at the limit of detection of cancer in man, say the amount of aflatoxin in the total diet which would induce one case of liver cancer per 10^6 population per annum. On the other, it can be argued that any level of cancer from aflatoxins is too high, so that the ALOP might just be lower than that by a factor of 1000 (ICMSF 2002).

The Food Safety Objective (FSO) concept has generally been applied to safety from pathogenic and toxigenic bacteria, but has been also applied to mycotoxins, as the maximum concentration of the hazard in a food at the time of consumption (ICMSF 2002; Garcia-Cela et al. 2012; Pitt et al. 2013). In the case of a chemical toxin such as aflatoxins, the limits set by a country for aflatoxins in foods can be logically considered also to have the status of a FSO (ICMSF 2002; Pitt et al. 2013).

During the mid-1990s, JECFA and CCFAC carried out a thorough re-examination of the toxicity of aflatoxins, especially in the light of newer evidence on the influence of hepatitis viruses on its carcinogenicity. CCFAC recommended to Codex that the maximum permitted level for total aflatoxins in foods in international trade should be 15 μg/kg. In 2009, Codex adopted this recommendation and it is accepted that FSO in this case is equal to that limit, and a FSO of 15 μg/kg has been established for peanuts for further processing in international trade (ICMSF 2002).

This FSO is based on a number of factors. First, statistical analyses of detailed surveys of the levels of aflatoxins occurring in European foods have shown that a reduction of that limit to 10 or even 5 μg/kg has only a marginal effect on the risk associated with aflatoxin consumption in importing countries. Second, it is difficult for producing countries to reliably supply nuts below 15 μg/kg to the consuming countries. Third, JECFA has stated that the evidence that aflatoxin is a confirmed Class I carcinogen in humans in the absence of hepatitis B virus remains inconclusive. This FSO may have to be adjusted downwards if conclusive evidence becomes available. This FSO is considered to be technologically achievable by major exporting countries, including the United States and Australia, but is currently out of reach of a number of producing countries in the tropics (ICMSF 2002).

14.3.3 Identifying and Establishing Performance Objectives

The FSO is preceded by the performance objective (PO), which is the maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption (ICMSF 2002), that provides or contributes to the achievement of a FSO. In case of mycotoxins, both

toxigenic fungi (which are not a biological hazard per se) and mycotoxins, as a chemical hazard, should be controlled.

POs are linked to the FSO and, when proposed by governments, can be viewed as a kind of milestone that governments provide as guidance to help meeting the FSO. For example, European Union Commission Regulation 1881/2006 and the Brazilian Sanitary Surveillance Agency (ANVISA) set certain mycotoxin limits for cereals and nuts for further processing which still have to undergo physical or other treatments before direct consumption (EC 2006; ANVISA 2011). In this case if moisture content of the product is maintained at low level, aflatoxin content does not increase and PO can be equal to FSO.

14.3.3.1 Distribution/Marketing

When processed peanuts are maintained at low moisture content (generally less than 10%) in the original package, it is expected that no changes occur in the aflatoxin content.

14.3.3.2 Manufacturing/Port of Entry

The Codex Alimentarius (2004) has a "Code of practice for the prevention and reduction of aflatoxin contamination in peanuts," which provides guidance for producing and handling peanuts for entry into international trade for human consumption. According to Codex Alimentarius (2004), post-harvest storage of peanuts is the phase that can contribute most to the aflatoxin problem in peanuts, due to condensation or leaks in the warehouse. Consignments of peanuts should be protected from all additional moisture by using covered or airtight containers or tarpaulins. Temperature fluctuations that may cause condensation to form on the peanuts, which could lead to local moisture build-up and consequent fungal growth and aflatoxin formation should be avoided. Water activity, which varies with moisture content and temperature, should be carefully controlled during storage. Prevention of aflatoxin increase during storage and transportation depends on keeping the low moisture content low, maintaining appropriate environmental temperatures, and ensuring hygienic conditions. *A. flavus/A. parasiticus* cannot grow or produce aflatoxins at water activities less than 0.7; relative humidity should be kept below 70% and temperatures between 0 and 10 °C are optimal for minimizing deterioration and fungal growth during long time storage. The aflatoxin level in peanuts coming into storage and peanuts going out of a storage should be monitored, using appropriate sampling and testing programs.

14.3.3.3 Ingredient

Nuts used in nut butters or finished blends without further processing should come from suppliers using GAP, GHP and GMP. Once peanuts are dried at safe level, moisture content of the product should be maintained at low level during storage to prevent fungal growth and aflatoxin formation. The content of aflatoxins in peanuts should not exceed 15 μg/kg or the limit established by the company. Once the final product is produced, it should be packaged properly to avoid water entry and keep its quality.

14.3.3.4 Primary Production

A careful sampling plan must be applied to assure that the PO is achieved. Codex Alimentarius (2001) designed a sampling plan for total aflatoxins in peanuts intended for further processing, using a single 20 kg laboratory sample of shelled peanuts (27 kg of unshelled peanuts) to be taken from a lot (sub-lot) and tested against a maximum level of 15 μg/kg for total aflatoxins. Each sample should

be composed of at least one hundred incremental samples, taken in a representative manner, using a systematic random sampling method, from locations throughout the lot. A hammer mill with 3.1 mm diameter hole in the screen is recommended for peanuts and a minimal test portion size of 100 g for comminuted peanuts. If larger test portions or mills that produce a finer grind are used to prepare the sample, a lower sample preparation variance will result. This sampling plan has been designed for enforcement and control concerning total aflatoxins in bulk consignments of peanuts traded in the export market (Pitt et al. 2012).

A sampling plan for the determination of aflatoxins in edible nuts was specified by European Commission Directive 98/53/EC (EC 1998a). For a batch of peanuts >15 metric tons, a 30 kg sample composed of 100 incremental samples is required. If the peanuts is not intended for further processing, the 30 kg sample must be mixed and divided into three 10 kg subsamples, these should then comminuted and mixed before the analysis. For unprocessed peanuts, the 30 kg sample should not contain more than 15 μg/kg for total aflatoxins. For those commodities intended for direct human consumption, the batch is accepted if each of the three 10 kg samples contains no more than 4 μg/kg for total aflatoxins (EC 1998b).

14.3.4 Performance Criteria

The performance criteria is the use of color sorting or other procedures as necessary to reduce the levels of aflatoxin in peanuts, until the results of assays on representative samples indicate that the acceptable level of <15 μg/kg have been achieved consistently. In Australia, one peanut shelling company sorts peanuts until the mean aflatoxin content of samples from any one lot does not exceed 3 μg/kg: this provides 95% confidence that any lot will meet the 15 μ/kg FSO (Pitt 2004).

14.3.4.1 Process Criteria

The major steps for reducing aflatoxins ($\sum$R) in shelled peanuts include: color sorting, blanching and roasting. Roasting peanuts is applied at various time-temperature combinations, as 150–160 °C for 30–120 min or 170 –185 °C for 8–11 min or higher temperatures such as 290 °C for 6–13 min. Thus the decrease in aflatoxins in peanuts during roasting may vary, although assays of representative samples indicated that the acceptable level of <15 μg/kg can be achieved consistently using the above processes. The extent of the reduction is dependent on the initial level of contamination, temperature, heating time and type of food (Arzandeh and Jinap 2011). Park and Liang (1993) showed the cumulative reduction of aflatoxin in peanuts using several processing steps as shown in Table 14.1. In this study a cumulative reduction higher than 99% was achieved. Other studies carried out on reduction of aflatoxin content in peanuts are shown in Table 14.2. Although differences exist among the studies, these data are useful for allowing peanut processors to use different control measures at one step or combination of steps to achieve the PO and FSO.

Table 14.1 Aflatoxin reduction at different steps of peanut processing (Park and Liang 1993)

Processing	Aflatoxin concentration (μg/kg)	Aflatoxin reduction (%)	Cumulative reduction (%)
Farmer stock	217	–	–
Belt separator	140	35	35
Shelling plant	100	29	54
Color sorting	30	70	86
Gravity table	25	16	88
Blanching/color sorting	2.2	91	99
Re-color sorting	1.6	27	99.3

Table 14.2 Aflatoxin reduction in manufacturing peanuts

Processing	Aflatoxin reduction (%)	Reference
Sorting	70	Dickens and Whitaker (1975)
Blanching	90	Whitaker (1997)
	27	Siwela et al. (2011)
	91	Park and Liang (1993)
Roasting	51 (160 °C)	Siwela et al. (2011)
	43–83 (150 °C/30 min)	Lee et al. (1969)
	30–45 (150 °C/30 min)	Pluyer et al. (1987)
	78–80 (150 °C/120 min)	Arzandeh and Jinap (2011)
	82 (150 °C/30 min)	Ogunswanwo et al. (2004)

Another process reported to decrease aflatoxin is soaking peanuts in acidified electrolyzed oxidizing water for 15 min at room temperature (85% reduction) (Zhang et al. 2012), although this is not applicable in the peanut industry. In Brazil nuts, processing which included manual or mechanical sorting and drying at 60 °C/30–36 h eliminated more than 98% of total aflatoxins, on average, showing that sorting is a very effective way to decrease aflatoxin content in Brazil nuts (Calderari et al. 2013). A study on pistachio nuts has shown a reduction of 30–50% during roasting, depending on the time-temperature applied, with an additional reduction of 26% during sorting (Garcia-Cela et al. 2012).

14.3.4.2 Product Criteria

At present, product criteria for aflatoxins in peanuts for further processing is <15 μg/kg, according to the international standard. Monitoring consists of controlling water activity at 0.7, and performing aflatoxin assays to ensure that the processing system in the shelling plant can consistently deliver sorted nuts with <15 μg/kg total aflatoxins. This system, which provides validation for the GMP/HACCP processes, is carried out on color sorted nuts either continuously from the process stream or using a recognized sampling plan. Aflatoxigenic fungi are not able to grow or produce aflatoxins at water activities less than 0.7. Avoiding the increase of the moisture content will ensure that aflatoxins are not produced in peanuts.

14.3.4.3 Shelf-Life Limits

The amount of aflatoxins in properly processed peanuts will not undergo changes. The end of the shelf-life of peanuts is more likely to be caused by rancidity, which can be controlled by roasting at higher temperature (Cammerer and Kroh 2009).

14.3.5 Acceptance Criteria

14.3.5.1 Microbiological

Although well standardized procedures exist for the examination of peanuts for the presence of *Aspergillus flavus* and *A. parasiticus* (Pitt and Hocking 2009), these are unlikely to find a place in international trade. However if the product or ingredient are shipped or stored at improper conditions, an increase on moisture content may occur leading to the development of aflatoxigenic fungi. Testing the product on the culture medium Aspergillus Flavus Parasiticus agar (AFPA) will distinguish the aflatoxigenic fungi from the others giving an indication of these toxigenic fungal growth (Pitt and Hocking 2009).

14.3.5.2 Chemical and Physical

The primary criterion for acceptance of peanuts for further processing in international trade is certification of total aflatoxins as <15 μg/kg. The Association of Official Analytical Chemists (AOAC 1984) official methods for aflatoxins in peanuts have been used in some countries as *de facto* standard method and these have been revised from time to time. For acceptable/not acceptable testing, immunochemical methods are most frequently used. For advanced users such as high volume analytical laboratories or regulatory authorities, liquid chromatography, sometimes coupled with mass spectroscopy has become normal practice. Limits of detection are now well below 1 μg kg^{-1}. Techniques on mycotoxin analysis have been updated elsewhere (Krska et al. 2008; Shephard et al. 2010).

Sampling procedures used by manufacturers to detect aflatoxins in incoming material is not 100% effective in rejecting all contaminated lots and in accepting all non-contaminated lots, because sampling and testing variation make discerning the real contamination level with 100% certainty impossible. Analyses of samples will yield an estimate of the contamination level, although sampling, sample preparation, analytical variation and the estimate of the level are subject to error. Generally, as the amount of sampling, sample preparation, subsampling, and analytical testing increases, a more precise estimate of the real lot concentration is obtained and fewer lots are misclassified by the sampling plan. However, increasing sampling plan is more costly, and the manufacturer is forced to compromise between cost and precision.

14.3.5.3 Organoleptic

Peanuts are graded on size and color for distribution in international trade.

14.4 Relating Risk Management Metrics to Stringency of Food Safety System

14.4.1 HACCP Plans

Aflatoxins in peanuts is both a microbial and a chemical issue. Control of aflatoxin production in the field is mainly achieved through minimizing the insect infestation and controlling the irrigation and the storage. During manufacturing, sorting and roasting are the main steps in the reduction of aflatoxin and thus potential critical control points.

14.4.2 Good Hygienic Practice (GHP) Programs

Farm management practice. Farm management practice has an important role in limiting aflatoxins in peanuts. Management of drought stress by irrigation is the best preventive measure, but most of the world's peanuts are grown under dry culture conditions where irrigation is expensive or impractical. Factors such as weed control, increased plant and row spacing and any other technique which increases water holding capacity in soils are important. Cultivars with reduced susceptibility to *A. flavus* infection have long been sought, but with little success. Other approaches include the possibility of using biological control, by applying atoxigenic isolates of *A. flavus* by competitive exclusion to reduce aflatoxin concentration in the crop. The technique, developed independently in the United

States and Australia, relies on the fact that only about 40% of *Aspergillus flavus* strains produce aflatoxins. Selected nontoxigenic strains that are both competitive in the field and unlikely to revert to toxicity are introduced, in high numbers, into soils in fields where peanut or cotton crops are being grown. The nontoxigenic spores compete with the existing toxin producing spores in the soil for infection sites on developing nuts. In sufficiently high numbers, control can be very effective (Cotty 1994; Pitt and Hocking 2006; Jaime-Garcia and Cotty 2007; Dorner and Horn 2007).

Improved drying practices, rapid threshing and mechanical drying are all valuable measures, but applicability varies widely with farm type. Adequate storage on farm is essential, which is a minor problem in developed economies such as the United States and Australia, but very difficult to implement in subsistence agriculture in the humid tropics.

Total control of aflatoxin formation in peanuts is difficult with current knowledge, primarily because in bad seasons, i.e. seasons with severe drought stress in the 2–3 weeks before harvest, aflatoxin forms in nuts before pulling from the soil. In regions where dry land farming is practiced and irrigation is impossible, good farm management cannot overcome this problem. Hence GHP practiced on farm can assist in aflatoxin reduction, but not in complete prevention.

The most important good farm practices are (CAC 2004):

- Maintaining soil moisture by weed control and other appropriate measures;
- Harvesting as early as possible, to reduce the time and severity of drought stress;
- Drying to safe moisture contents (0.70 a_w, equivalent to 8% moisture) as rapidly as possible, either in the field or by mechanical means;
- Storing at constant temperatures, in well-designed bins, in shade, and with good moisture control, preferably with monitoring of humidity;
- Cleaning before transport to shellers, to removal shriveled and damaged nuts more likely to contain high levels of aflatoxins.

Shelling plants Shelling plants, which generally control commercial peanut handling after harvest, have good storage practice for shelled peanuts in developed countries, but in developing countries the practices often leaves much to be desired. Transport of peanuts also may cause problems due to moisture migration.

Major GMP measures in the shelling plant include (CAC 2004):

- Random sampling at intake, to assess moisture and aflatoxin content – loads with excess moisture should be rejected and returned to the farm for drying, loads with excess aflatoxin should be segregated;
- Careful storage with control of insects, temperature gradients and moisture, as on farm;
- Color sorting after shelling, preferably using instruments which can distinguish more than one color, and which can be set to segregate larger or smaller proportions of the nuts dependent on the aflatoxin status of the raw material;
- Aflatoxin assays, carried out with an adequate sampling plan, preferably performed routinely.
- As necessary, roasting and blanching before color sorting and re-assay.
- Acceptable product should be stored under carefully controlled conditions until shipped.

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Chapter 15
Pathogens and Indicator Organisms in Powdered Infant Formula

15.1 Introduction

As defined by Codex Alimentarius (CAC 1981), infant formulae are breast-milk substitutes specially manufactured to satisfy, by themselves, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding. The international standard for powdered infant formula and formulae for special medical purpose has been established by Codex Alimentarius in 1981. It has since then been continuously reviewed to take into account the results of new medical research. Definitions as well the age group for which these products are intended may vary depending on the countries. In the case of Europe, for example, infant formulae are intended for infants up to 6 months of age, while follow-up formulae are designed for infants from 6–12 months (EC 2006). In the case of the United States, however, no differences are made and infant formulae are intended for infants from birth up to 12 months.

Powdered infant formula (PIF) are produced using one of the following processes:

1. **Wet-mix processes** during which all unprocessed raw materials and separately processed ingredients are mixed to obtain a liquid intermediate product, which is heat-treated, dried and then further handled up to the filling stage. In this process, no further additions are done after the heat-treatment and the drying step.
2. **Dry-mix processes** during which all separately processed ingredients are dry-blended to obtain the final product, which is then further handled up to the filling stage. The process may include and combine different mixing steps to obtain the final formula.
3. **Combined processes** during which part of the unprocessed raw materials and part of the ingredients are processed according to the wet-mix process to obtain a base powder. This base powder is considered as an intermediate product and then further used for the manufacture of different finished products by the addition of separately processed ingredients.

Historically, *Salmonella* was long considered the only significant pathogen in PIF. Following sporadic outbreaks with *Cronobacter* spp., formerly known as *Enterobacter sakazakii,* which caused severe health effects in infants, three FAO/WHO expert consultations (FAO/WHO 2004, 2006, 2008a) were organized to carry out risk assessments. Both organisms were confirmed as relevant pathogens with clear evidence in causality. This resulted in the issuance of a revised Codex Code of Hygienic Practice for Powdered Formulae for Infants and Young Children which includes microbiological criteria for PIF and "follow-on" formulae (CAC 2008).

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While several studies showed no mathematical correlation between the presence of *Enterobacteriaceae* and *Salmonella* or *Cronobacter* spp. (FAO/WHO 2006; EFSA 2007), their use as hygiene indicator during the manufacture of powdered infant formulae is strongly supported in the conclusions of the FAO/WHO report (2006) as "the ideal tool to assess the effectiveness of preventive measures and to detect the occurrence of recontamination" (*sic*). As a consequence, stringent requirements for *Enterobacteriaceae* as a hygiene indicator have been included in the microbiological criteria published by Codex Alimentarius (CAC 2008).

Aerobic mesophilic bacteria, a parameter already part of the previous microbiological criteria, have been maintained and tightened in the current criteria (CAC 2008). They are based on empirical and historical experience and used as indicators to verify adherence to Good Manufacturing Practices in the wet part of the process up to the spray dryer. The parameter provides useful information on the build-up of bacteria in equipment such as evaporators or contamination due leaks in plate heat exchangers used to process the liquid base.

15.2 Risk Assessment

15.2.1 Hazard Identification

Powdered infant formulae contaminated with *Salmonella enterica* have been historically linked to occasional outbreaks, including several recent outbreaks (Usera et al. 1996, 1998; Brouard et al. 2007; Jourdan et al. 2008; Rodríguez-Urrego et al. 2010). Historical data are summarized and discussed by FAO/WHO (2006), Cahill et al. (2008) and ICMSF (2011). *Cronobacter* spp. has been linked to rare but severe cases and outbreaks. Around 30% of these have been directly linked to intrinsically, contaminated infant formulae. Other investigations pointed to contamination during preparation or in several cases, showed that infants had not been fed with PIF. Reviews have been published in several reports and by several authors (FAO/WHO 2004, 2006, 2008a; Norberg et al. 2012). Numerous studies have been devoted to the prevalence of *Cronobacter* spp. in foods and environments and several authors have demonstrated that this organism is ubiquitous and present in numerous types of foods and environments where foods are prepared and handled (Turcovsky et al. 2011; Putthana et al. 2012; Belal et al. 2013).

In recent years several cases have been reported where the cause of the outbreak could not be attributed to intrinsically, contaminated infant formulae. In these cases infants had either been fed with breast-milk, ready-to-feed (sterile) formulae or mixtures thereof or no *Cronobacter* spp. could be found in infant formulae despite of thorough investigations. Considering the ubiquitous occurrence of *Cronobacter* spp. described above, contamination occurring during preparation and handling is a likely cause (Aguirre-Conde et al. 2007; Ray et al. 2007; Savage et al. 2008; Vanegas et al. 2009; CDC 2011, Broge and Lee 2013). In a review of *Cronobacter* spp. infections, Jason (2012) determined that between 2004–2010, there was an increase in cases involving non-hospitalized and term infants.

Both *Salmonella* and *Cronobacter* spp. have been included in category "A", i.e. as pathogens being relevant for powdered infant formulae due to a clear evidence in causality (FAO/WHO 2004, 2006) and no other pathogen has been rated "A". While *Salmonella* is considered a pathogen for infants as well as for young children and of course for consumers belonging to other age ranges, *Cronobacter* spp. has only been clearly established as pathogenic for infants up to 6 months with neonates (< 28 days), particularly pre-term infants, low-birth weight infants or immune-compromised infants (FAO/WHO 2004, 2006) presenting the greatest risk of illness. The FAO/WHO report (2008) specifically reviewed the situation for follow-up formula and concluded that there was no epidemiological evidence that infants beyond 6 months are susceptible. Publications reporting cases of *Cronobacter* spp. in adults as well as in the elderly are related with infections such as eye infections, urinary tract infections and other types of systemic infections, hence not foodborne (Lai 2001; Dümen 2010).

15.2.2 Exposure Assessment

Despite the occurrence of sporadic cases and outbreaks, it is reasonable to assume that the presence of *Salmonella* and *Cronobacter* spp. in products is infrequent and hence exposure is very low. Exposure to significant levels at the moment of consumption is mostly due to rehydrated products being improperly handled after reconstitution (Pagotto et al. 2003; FAO/WHO 2004, 2006).

In the case of *Salmonella* outbreaks linked to powdered infant formulae, published case studies have invariably shown deviations from good hygienic practices leading to the detectable presence of *Salmonella* in lots manufactured during a restricted period of time. Apart from these specific cases it can certainly be assumed that salmonellae are only present, if at all, at extremely low levels and therefore not detectable even by applying the most stringent sampling plans.

Products with low levels of *Cronobacter* spp. (<3 MPN/ 100 g) have been involved in outbreaks (FAO/WHO 2006). It must, however, be considered that inappropriate storage of reconstituted formula has been reported in several outbreaks, hence likely to have permitted growth and therefore exposure to high levels in individual servings. Data on prevalence of *Cronobacter* spp. in finished products are largely based of the analysis of products manufactured before the issuance of the revised microbiological criteria (CAC 2008). Based on data collected before 2006, concentrations of *Cronobacter* spp. in infant formulae were estimated to range between $10^{-5.24}$ and $10^{-2.79}$ CFU/g (FAO/WHO 2006).

The performance of the microbiological criteria for *Salmonella* and *Cronobacter* spp. published by Codex Alimentarius (CAC 2008) are expressed as Log mean concentration at which a lot will be rejected with 95% confidence. These means range between 1 in 590 g and 1 in 7400 g for *Salmonella* and between 1 in 120 and 1 in 980 g for *Cronobacter* spp., depending on the standard deviation of dispersion of the microorganisms within the lot. These values do not however allow conclusions to be reached on prevalence in commercialized products. While Dewanti-Hariyadi et al. (2010) concluded on a significant improvement of the management of PIF by manufacturers in Indonesia, no recent systematic detailed investigation on concentrations of *Cronobacter* spp. in PIF has been performed to determine the impact of new microbiological criteria with 100–1000 times stricter hygiene requirements compared to those enforced between 1979 and 2008, namely n = 5, c = 1, m < 3, M = 20, with a performance of around 3 CFU/g.

Based on a geometric mean concentration of $10^{-3.84}$ CFU/g and a worldwide production of 4.8×10^5 tons (FAO/WHO 2006; Cordier 2008), Reij et al. (2009) estimated a minimal number of *Cronobacter* spp. that is ingested, thus corresponding to an exposure, by all infants through PIF of 7.84 log CFU/year. Taking into account a daily consumption of 83 g of PIF (6 bottles of 100 mL containing on average of 13.8 g each) the authors estimated an exposure of individual infants of 0.335 CFU during the neonatal period of 28 days. The application of stricter hygiene control measures as advocated by CAC (2008) and described by Cordier (2008) would in principle lead to a reduction of the geometric mean concentration by a factor 100–1000 and hence to an equivalent reduction of the exposure.

15.2.3 Hazard Characterization

The common signs and symptoms of salmonellosis include diarrhea, bloody diarrhea and fever. Compared to adults, a higher percentage of infants require hospitalization to address excessive fluid losses. Severe symptoms such as meningitis or mortality occur rarely, if at all, in cases associated with PIF (Brouard et al. 2007; Rodriguez-Urrego et al. 2010).

Cronobacter spp. is a rare cause of illness, with a total of about 150 cases being reported over a period of 40–45 years. Disease syndromes include septicemia, necrotizing enterocolitis, or meningitis, with high rates (40–80%)%) of mortality or chronic sequelae (FAO/WHO 2004, 2006). Pre-term infants, low birth weight (< 2500 g) as well as immune-compromised infants are considered as those having the highest risk of disease.

15.2.4 Risk Characterization

The annual world production of PIF was estimated in 2005 at about 10^6 tons based on Euromonitor estimates (Cordier 2008), while US$41 billion was spent on milk formula globally in 2013 (Kent 2014). In the case of *Salmonella* outbreaks, often no details are provided on the type of products involved. Products such as follow-up formulae need to be included as well as products consumed by young children, who are also susceptible to *Salmonella,* hence resulting in considerably higher tonnages of products.

No dose-response data or model seem to exist for infants but existing models for other age groups indicate that illness can result from very low doses of salmonellae cells. Due to the lack of data, FAO/WHO (2006) assumed that illness could result from 1 CFU of *Cronobacter* per serving at the time of preparation and described the dose-response as an exponential model:

$$P_I = 1 - e^{(-rD)}$$

where *r* is the exponential dose-response parameter and *D* is the dose at consumption that results from an initial contamination level of 1 CFU of *E. sakazakii* per serving (prior to storage and growth) in the dry product. Reij et al. (2009) estimated the probability of infection during the neonatal period to range between 1.2×10^{-10} and 2×10^{-5}.

15.3 Risk Management

15.3.1 Control Measures

In addition to contamination during processing, and considering the causes of several of the reported outbreaks, the revised Code of Hygienic Practices for Infant Formulae and Food for Special Medical Purposes (CAC 2008) considered two additional potential routes of contamination; (1) after opening of the package and (2) during or after reconstitution by the caregiver due to the ubiquitous occurrence of *Cronobacter* spp., including hospitals, institutions, day-care facilities and homes (Kandhai et al. 2004; Carletti and Cattaneo 2008; Palcich et al. 2009; Kilonzo-Nthenge et al. 2012; Siqueira-Santos et al. 2013). This Code of Hygienic Practices therefore considers not only the need to apply control measures during manufacture but also during preparation and after reconstitution.

15.3.1.1 Primary Production

Not applicable

15.3.1.2 Manufacturing/Processing

The usual processing conditions applied during the heat-treatment (CCP) range from pasteurization (71–74 °C for 15–25 s) to sterilization applying direct steam injection (130 °C for at least 1–2 s). Since both organisms are heat sensitive, these processes achieve reductions of around 6–7 log units for the mildest treatments and far in excess of 50 log units for the more severe ones.

The presence of both *Salmonella* and *Cronobacter* spp. in finished products manufactured according to the three types of processing outlined in 15.1. are due to (a) the quality of the ingredients used to manufacture the products and (b) the contamination from the processing environment, along the whole process for exclusively dry-mixing operations or after the heat-treatment and drying in the case of wet-mixing or combined operations.

Cronobacter spp. show a very high degree of resistance to desiccation and is found to survive for prolonged periods of time in dry processing environments and in dry products. Although probably not as resistant, *Salmonella* is nevertheless also able to survive in dry conditions for prolonged periods of time. Post-process contamination after the kill step, contaminated processed ingredients added after the kill step, and processing environments and lines represent the main sources of these microorganisms in the finished products (FAO/WHO 2004, 2006; Cordier 2008).

The control measures to prevent contamination during further processing (post heat-treatment) are based on four general principles: (i) avoiding ingress into processing facilities and in particular into the high hygiene areas which comprise all processing steps from the dryer to filling; (ii) avoiding multiplication in these areas in case of ingress; (iii) avoiding establishment of niches in such areas and (iv) use of dry-mixed ingredients complying with the same requirements as those of the finished products.

Since *Cronobacter* spp. are much more ubiquitous than *Salmonella*, their control is far more difficult and tightened control measures aim at limiting additional ingress but are focused specifically to address (ii) and (iii) above. Detailed discussions on the control measures for both pathogens during manufacture are provided in Cordier (2008).

Since the control of *Salmonella,* and particularly *Cronobacter* spp., requires the application of extremely strict hygiene control measures, the verification of their effectiveness plays an important role. The importance of this parameter is illustrated by the establishment of a hygiene criterion for *Enterobacteriaceae* (n = 10, c = 2, m = 0 in 10 g) which is 100–1000 times stricter than the one for coliforms in the previous version of the Code of Hygienic Practice (n = 5, c = 1, m < 3, M = 20). Verification of control measures is best achieved by combining testing of environmental and in-process samples as outlined in Table 15.1. The use of microbiological indicators to verify the effectiveness of hygiene control measures during the manufacture of powdered infant formulae has been discussed by Buchanan and Oni (2012).

15.3.1.3 Distribution/Marketing

PIFs have low water activities (< 0.4) and no growth will occur during storage and distribution up to the final users. However, *Salmonella* and *Cronobacter* spp., if present, are able to survive in dry products over prolonged periods of time (Edelson-Mammel et al. 2005; Dancer et al. 2009; Walsh et al. 2011).

15.3.1.4 Preparation/Handling

Taking into account that poor hygiene and inappropriate handling and storage have been identified as causes in numerous reported cases, the Codex Code of Hygienic Practice considered that various combinations of control measures were suitable to achieve a significant risk reduction at the consumer level. These are immediate feeding after reconstitution, rapid cooling to the appropriate feeding temperature and the recommendations of the WHO to reconstitute formulae at a temperature of 70 °C. Several Public Health Authorities have issued national guidelines in which different recommendations for the preparation can be found, for example AFSSA (2005), WHO/FAO (2007) and Turck et al. (2012).

Table 15.1 Testing of powdered infant formulae for microbiological safety and quality

–	Relative importance	Useful testing							
Critical ingredients	High	It is important to develop good supplier relationships for critical dry mix ingredients to ensure their safety. Requirements should be equivalent to those for finished products (see below). Depending on the confidence level in the supplier, test either for acceptance or as monitoring							
In-process	High	Routine in-process testing is recommended at critical steps of the process. Requirements include:							
		Salmonella – absent in any sample ≥25 g							
		C. sakazakii – absent in any sample ≥10g							
		Enterobacteriaceae – absent in any sample of ≥10g							
Processing environment	High	Due to its widespread occurrence at very low levels, routine testing for *C. sakazakii* is not recommended. It may be considered for mapping of the situation in the plant or for investigation. Routine testing for *Salmonella* and Enterobacteriaceae is recommended							
		Salmonella – absent							
		Enterobacteriaceae – <10 CFU/g							
Shelf life	–	Not applicable							
End product	High	Test for indicators for on-going process control and trend analysis							
		Product	Microorganism	Analytical method[a]	Case	SSampling plan and limits/g[e]			
						n	cc	mm	MM
		Infant formula and Follow-up Formula	Aerobic colony count	ISO 4833	2[b]	5	2	5×10^2	5×10^3
	High					Sampling plan and limits/10 g[e]			
			Enterobacteriaceae	ISO 21528–1	Codex[b]	10[c]	2	0	–
	High	When in-process and environmental results are negative for *Salmonella*, testing a limited number of samples for verification is usually sufficient. When these data indicate a potential for contamination or when the effectiveness of control measures seems impaired (e.g., construction activities, wet cleaning), test according to recommendations below Considering that *C.sakazakii* is much more widespread and even if controlled to very low levels in the environment, testing according to the plans below for lot acceptance is recommended							
		Product	Microorganism	Analytical method[a]	Case	Sampling plan and limits/25 g[e]			
						n	c	m	M
		Infant formula and Follow-up Formula Infant formula	*Salmonella*	ISO 6785	15[b]	60[d]	0	0	–
	High		*Cronobacter* spp	ISO TS 22964	14[b]	Sampling plan and limits/10 g[e]			
						30[c]	0	0	–

[a]Alternative methods may be used when validated against ISO methods
[b]Codex Alimentarius (2008) criteria are recommended
[c]Individual 10 g analytical units
[d]Individual 25 g analytical units
[e]Refer to Appendix 1 for performance of these sampling plans (ICMSF 2011)

While reconstitution at 70 °C can achieve a reduction of about 4 log units of vegetative cells, the initial risk assessment (FAO/WHO 2004) also considered the overall risk mitigation would be less if only 80% of the users followed this procedure. Several publications have highlighted weaknesses in the application of good hygiene practices in locations where such formula are prepared (Renfrew et al. 2008; Redmond et al. 2009; Calamusa et al. 2009; Dumm et al. 2010) including the use of tap water without previous boiling as recommended by manufacturers (Kozlica et al. 2010). The occurrence of several cases linked to the use of sterile ready-to-feed formulae which is considered as one of the safest options, and a fatality in Japan (Teramoto et al. 2010), where reconstitution at 70 °C is a mandatory requirement, clearly indicate that good hygiene practices during preparation and handling cannot be neglected.

Adherence by users to guidelines provided to ensure the safe preparation of reconstituted formulae is an integral part of the overall food safety system for PIF by users.

15.3.2 ALOP/FSO

While no formal Food Safety Objective or Performance Objective have been established for powdered infant formulae, the log mean concentration needed to consistently meet the established microbiological criteria for *Salmonella* (n = 60, c = 0, m = 0 (in 25 g)) and *Cronobacter* spp. (n = 30, c = 0, m = 0 (in 10 g)) could be considered as such. Such log mean concentrations would certainly have to be in the order of 10^{-5} CFU/g or lower for *Cronobacter* spp. and 10^{-6} CFU/g or lower in the case of *Salmonella* to achieve these targets.

15.3.3 Identifying and Establishing Performance Objectives

15.3.3.1 Primary Production

Not applicable

15.3.3.2 Manufacturing

Considering that no growth is possible in the powder after the drying step, the Performance Objective at the end of manufacturing can be considered equivalent to the Food Safety Objective.

15.3.3.3 Distribution

Considering that no growth is possible in the powder after the drying step, the Performance Objective during distribution and up to the consumer can be considered equivalent to the Food Safety Objective.

15.3.3.4 Preparation

Equivalent to the Food Safety Objective mentioned in Sect. 15.3.2. This assumes that the infants' caregiver prepares and feeds the product in a manner that prevents growth of *Salmonella* or *Cronobacter* spp.

15.3.4 Performance Criterion

A performance criterion is the required outcome of a step, or combination of steps, that contribute to ensure that the PO/FSO is achieved. In the case of powdered infant formulae the reduction is ensured by a heat-treatment with different performances ranging from a reduction by 6–7 log units to more than 50 as outlined in Sect. 15.3.1.2.

15.3.5 Acceptance Criteria for Final Product

15.3.5.1 Organoleptic

Each type of PIF has particular organoleptic characteristics depending on its composition and certain of these parameters are used for release.

15.3.5.2 Chemical, Physical and Nutritional

PIF must comply with the requirements laid down in the Codex Alimentarius Standard (CAC 1981) for these products or in relevant national regulations, where existing. These requirements are, on one side, related to the nutrient composition and, on the other side, to criteria for chemical contaminants.

15.3.5.3 Microbiological

In the case of manufacturers focusing on the application of an integrated sampling plan with in-process and environmental samples, end product testing for *Salmonella* is usually limited and only performed for verification (ICMSF 2011). Positive results of either in-process or environmental samples indicating a potential for increased risk of presence of *Salmonella* in the finished product, should trigger a change in the sampling regime, i.e. testing of up to 60 analytical units of 25 g for release purposes may become appropriate under such conditions (see Table 15.1) (ICMSF 2011). Considering the widespread occurrence of *Cronobacter* spp. in processing environments, even if it can be controlled to a low to very low levels, testing of finished products has a higher importance and is therefore frequently applied for release to confirm compliance with regulatory requirements.

During the revision of the Codex Alimentarius Code of Hygiene for Infant Formulae, the ICMSF proposed a 2-class plan for *E. sakazakii* (*Cronobacter* spp.) based on the FAO/WHO risk assessments (FAO/WHO 2004, 2006). This 2-class plan was adopted by Codex Alimentarius (2008) and is applied or considered in several other national regulations.

For indicators, a change from coliforms to the more precisely defined *Enterobacteriaceae* is recommended based on the outcome of the two expert meetings (FAO/WHO 2004, 2006). Much more stringent requirements than the criteria in the former Code of Hygiene (i.e., for coliforms n = 5, c = 1, m < 1 CUF/g, M = 20 CFU/g) are now considered appropriate to reflect the increased risk of contamination with *Cronobacter* spp. Such stringent criteria (i.e., for *Enterobacteriaceae* n = 10, c = 0 or 2, m = 0 in 10 g samples) have been implemented in the EU and in other countries (EC 2007).

An expert consultation reviewed existing scientific and technical information on the relevance of *Cronobacter* spp. for follow-up formulae and based on the lack of evidence, criteria were limited to *Salmonella* and *Enterobacteriaceae*, with no limits set for *Cronobacter* (FAO/WHO 2008a).

Jongenburger et al. (2011) have compared the performance of different sampling strategies on naturally contaminated lots of infant formulae. They showed that more and smaller samples (for an

equivalent total weight) and a stratified sampling improved the performance of the sampling plans in detecting a heterogeneous contamination. Sampling and testing of $n = 30$ (in 10 g) according to the criterion defined by Codex Alimentarius (CAC 2008) showed the best performance with a probability of 97% of yielding at least one positive sample in the case of random sampling and 98.5% in the case of stratified sampling. All other sampling plans evaluated provided significantly lower probabilities of detection. It can certainly be concluded that the established criterion fulfills the intended purpose of detecting and blocking lots which might lead to cases of illness.

15.4 Relating Risk Metrics to Stringency of Food Safety System

15.4.1 HACCP Programs

The step at which the heat-treatment is applied during processing is considered as CCP and needs to be managed as such. Critical limits may vary depending on the type of heat-treatment applied.

Considering the stringency of the hygiene control measures required for control of *Salmonella,* and particularly *Cronobacter* spp., other control measures, usually considered as pre-requisite programs such as the air handling units, may be considered as operational prerequisite programs (OPRPs) or CCPs and managed as such.

15.4.2 GHP Programs

The manufacture of PIF complying with the strict microbiological criteria published by Codex Alimentarius (CAC 2008) requires far stricter pre-requisite programs than what was necessary in the past for control of *Salmonella*. The main difference resides in a much stricter control of the presence of traces of water or humidity to prevent growth of *Cronobacter* spp. present at low levels in processing environments. Details on the history of the development of hygiene control measures to manufacture PIF as well as the differences in the management of *Salmonella* or *Cronobacter* spp. have been described in details by Cordier (2008).

15.4.3 Regulatory Requirements and Criteria

Microbiological criteria for PIF have been included in the Code of Hygienic Practices (CAC 2008). In addition several national or supranational regulations have been established such as the European Community (EC 2005 2007) or China (2010) criteria.

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Chapter 16
Listeria monocytogenes in Ready-to-Eat Deli-Meats

16.1 Introduction

Listeria monocytogenes is an important foodborne pathogen which is widely distributed in nature and can be found on almost all foods, in soil, water, sewage, silage, slaughterhouse waste, milk from healthy and mastitic cows, as well as in human and animal feces (Farber and Peterkin 1991, 1999; Sauders et al. 2006, 2012). It is among a small number of foodborne pathogens that are capable of growth at low temperatures, to survive for very long periods of time in food processing facilities and its association with a high case-fatality rates. Although foodborne listeriosis occurs infrequently, at somewhere between 2 and 6 cases annually per million of population, between 20 to 30% of the cases are fatal (McLauchlin 1993; Rocourt 1996; Mead et al. 1999; Silk et al. 2012).

This chapter considers *L. monocytogenes* in ready-to-eat (RTE) deli meats as an example of a microbial hazard that is capable of growth in a wide variety of perishable RTE foods. The use of performance objectives, performance criteria, product and process criteria, and validation in relation to HACCP plans will be described. In this example as in many food, this pathogen is psychotrophic, establishes itself in niches and/or biofilms in food processing plants, and multiplies in the refrigerated areas of the food operation and in the refrigerated food. This chapter describes the application of principles introduced in previous chapters. Hypothetical values have been used throughout the chapter wherever assumptions were necessary to illustrate a concept or procedure. No attempt has been made to verify their accuracy.

A wide range of cooked meat products are produced across the world, using different meat species, ingredients, and cooking processes. Cooked meats are described in different countries as deli meats, luncheon meats, and small goods, but some of these categories may also include uncooked (for example, fermented) products. The meat may be beef, pork, lamb, chicken, turkey, other meats or mixtures. They may be whole muscle products, such as ham or corned beef, emulsion products packed in casings which may or may not be removed after cooking (e.g., frankfurters, pepperoni) or fabricated products (e.g., head cheese). The current chapter will focus on deli-meats. Cooked deli-meats are RTE and normally consumed without further listericidal steps (CAC 2007c).

Deli meats such as ham, roast beef, and smoked turkey breast all have very similar processes, and are produced by adding a solution of ingredients to the raw meat ingredient. Cured products, like ham, turkey ham, and corned beef, have nitrite in the solution. Other products, such as roast beef or chicken roll, may only contain salt and seasonings. The solution is often added with an injector, but products may also simply be immersed in the solution. Products can be tumbled or massaged, which increases

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both yield and tenderness. This produces muscle fiber disruption, with a corresponding release of salt-soluble protein, which in turn coats the meat pieces. The protein is then coagulated by cooking to form a matrix between the individual pieces, thus giving the product an intact muscle appearance. Meat pieces can be formed into uniform shapes by placing them into nets, casings or molds.

The cooking and cooling of these products is similar to that for cooked sausage products. During cooking, the parameters that must be controlled are temperature, time, and humidity. The product must be exposed to a high enough temperature to produce a fully cooked, RTE product. The temperature inside the smoke house, and the internal temperature may be monitored to verify that the critical limits are met. Cooking is a very important step, because it is here that any pathogens that may be in the product will be eliminated and the numbers of spoilage bacteria will be lowered to an acceptable level. After the product has reached the final desired temperature, the cooling process begins which often involves showering with cold water inside the smokehouse. This removes some of the heat from the product, and immediately halts the cooking process. The shower is usually not sufficient to complete the cooling process, and to finish the cooling process, products are usually moved to another chiller/cooler. Some operators use cold water for chilling, sometimes with salt added to lower the temperature below the normal freezing point of water. This is called a brine chiller. Other establishments may use cold air, and some use a combination of methods. Some of these products, however, are cooked in a water bath or in a steam chamber. After chilling, many of the products are packaged as, e.g., whole roasts, for the retail deli market, while others are sliced and packaged in retail consumer sized portions. Many are vacuum-packaged, which helps to protect the product quality and increase the shelf-life.

Adequate cooking renders meats free of *L. monocytogenes*. However, post-processing contamination can occur and has been the cause of a number of large meat-borne listeriosis outbreaks, such as the Canadian outbreak associated with the consumption of deli-meats, in which 24 deaths occurred. (Currie et al. 2015). It should be noted that multiplication of *L. monocytogenes* on cooked deli-meats is generally considered necessary for disease to occur. There is limited data on the dose-response relationship of *L. monocytogenes* in humans and, unfortunately, suitable animal models of infection are still lacking (Williams et al. 2009; Hoelzer et al. 2013). Recent listeriosis outbreaks associated with meat and poultry can be seen in Table 16.1.

16.2 Risk Evaluation

16.2.1 Published Risk Assessments/Risk Profiles

A number of risk assessments dealing with *L. monocytogenes* and meat/poultry products have been conducted (Table 16.2) ranging from through-chain risk assessments (Bemrah et al. 1998) to estimates of risk across a wide range of foods at the time of consumption (FDA/FSIS 2001, 2003), to risk assessments at the retail level (Endrikat et al. 2010).

L. monocytogenes is inactivated by heat treatments of 70 °C, a temperature that is typically achieved by most cooking or pasteurization interventions. Meat products such as deli-meats can be re-contaminated between cooking and packaging in the processing facility. During extended refrigerated storage (e.g., ≥ 50 days), a surface recontaminated deli-meat product could support the growth of the organism to potentially hazardous levels on the surface of the meat, especially if the product is temperature-abused and does not contain any growth inhibitors. The free exudates in the package sometimes have a higher concentration of cells than the product itself, and may be a source of cross-contamination in the kitchen. Products are usually eaten without further cooking. Deli meats can also be re-contaminated at the retail level due to operations such as slicing or cross-contamination in the display cases.

Risk assessments of RTE meat products, including cooked meats have been published (Ross et al. 2009a, b, c, d). A listing some of the recent risk assessments along with a short description of the major findings can be seen in Table 16.2.

Table 16.1 Listeriosis outbreaks since 2009 linked to meat and poultry

Vehicle	Year	Subtype	Illnesses (Deaths)	Country	Reference
Rolled deli sausage	2013/14		20 (12)	Denmark	http://www.ssi.dk/English/News/News/2014/2014_08_listeria.aspx
Salami	2010		2	Canada	Foodborne illness outbreak Database (2010)
Chicken wrap	2009		13 23 non invasive (3 fetal)	Australia (Virgin flight)	OzFoodNet Working Group (2009)
Cooked ham	2011	1/2a	9	Switzerland	Hächler et al. (2013)
Sandwiches	2010	4	5 (1)	UK	Little et al. (2012)
Sandwiches	2011	4	3 (0)	UK	Little et al. (2012)

Table 16.2 Listing of risk assessments- and risk-associated publications

Product/s	Extent of the supply chain	Notes	Reference
A total of 23 ready-to-eat foods in the USA, including frankfurters, dry/semi-dry fermented sausages, cooked, ready-to-eat meats, pâté and meat spreads	Risk at the time of consumption – per serving and per annum		FDA/FSIS (2003)
The review is written to provide a clinical and epidemiological background to the mathematically oriented, as well as to outline the mathematical approaches to those interested in food-borne infection.	Adverse health effects, together with incidence data on different age and risk groups for human *L. monocytogenes* infections	Hazard characterisation- the qualitative and/or quantitative evaluation of the adverse health effect associated with the hazard	McLauchlin et al. (2004)
Milk, ice cream, smoked fish, fermented meats	Retail to consumption		FAO/WHO (2004)
RTE meats		Dose response	Walls (2006)
Cured cooked sausages ready-to-eat, fermented sausages, deli meats, pâté, fresh sausage	Raw material to consumption	Semi-quantitative assessment	Sumner et al. (2005)
Ham, dry cured ham	Bone-in, de-boned and sliced hams, near end of production chain	Exposure assessment Dose response assessment	Giovannini et al. (2007)
Pork and poultry meat		Risk ranger. It is a semi-quantitative risk estimator	Mataragas et al. (2008)
Ready-to-eat meats in Australia-cooked meats, pâté, cooked sausages	Post-cooking to consumption	Risk assessment	Ross et al. (2009a, b, c, d)
Ready-to-eat meats in Australia-cooked meats, pâté, cooked sausages	Post-cooking to consumption	Risk-reduction mitigations modelled	Ross et al. (2009a, b, c, d)
Smoked fish (salmon and trout) and sliced cooked ham (vacuum or non-vacuum-packed)		Rick characterization/ what if scenarios	Garrido et al. (2010); Pouillot et al. (2007); Pouillot et al. (2009)
Sliced, cooked, cured ham-like meat	Sliced product to consumption	Sensitivity analysis; consider PO	Mataragas et al. (2010)
Retail-sliced versus prepackaged and with or without growth inhibitor; deli meat and poultry	Retail stage, growth stage, consumption stage, dose-response stage	Risk assessment	FSIS (2010)
Deals with the consumption of ready-to-eat foods commonly prepared and sold in the delicatessen of a retail food store.	The quantitative risk assessment (QRA) model simulates the behavior of retail employees in a deli department and tracks *L. monocytogenes* potentially present in this environment and in the food	The risk assessment model predicts that cross-contamination with *L. monocytogenes* at retail predominantly results in sporadic cases	Pouillot et al. (2015)

16.2.2 Exposure Assessment

Exposure assessment is one of the four components of a risk assessment within the risk analysis framework adopted by Codex as the basis for standard-setting processes. Exposure assessment includes an assessment of the extent of actual or anticipated human exposure. For microbiological agents, exposure assessment is typically based on the potential extent of food contamination by a

particular agent or its toxins, conditions between consumer purchase and consumption, and dietary information related to frequency and extent of consumption.

L. monocytogenes is found as a frequent contaminant of RTE meats (Farber and Peterkin 1991, 1999; Gombas et al. 2003; Gambarin et al. 2012), and there have been a number of listeriosis outbreaks linked to RTE meats (Ho et al. 1986; Kittson 1992; McLauchlin et al. 1991; CDC 1999). Notwithstanding, the meat industry has a long history of providing safe meats. Wallace et al. (2003) found *L. monocytogenes* to be present in 1.6% of packages of frankfurters, and USDA/FSIS detected it in 3.25% samples in dry and semi dry fermented sausage, over a 10 year period (1990–1999) (Levine et al. 2001). The USDA monitoring program for RTE meats after packaging at the manufacturing facility has found the prevalence of *L. monocytogenes* in RTE meats and poultry to have decreased substantially over the last 20 years, i.e., an observed incidence of 4.61, 1.45 and 0.32% in 1990, 2000 and 2010, respectively (FSIS 2012).

Raw materials

The higher the concentration of *L. monocytogenes* on the incoming raw materials, the more effective pathogen control processes need to be to reduce concentrations to acceptable levels. In addition, high microbial loads on raw meat entering the processing plant increase the potential for contamination of the processing environment and potentially the finished product. *L. monocytogenes* is generally found at low levels in raw meat and other raw materials and products can become re-contaminated post-thermal processing.

Cooking

Cooked meat processing involves a heating schedule which varies according to product types (emulsion and whole muscle) Cooking processes generally are accepted as eliminating the maximum levels of *L. monocytogenes* (~10^3 CFU/g) that could conceivably occur in raw materials. In addition, "post-pack pasteurization"(e.g., thermal or high-pressure processing) can eliminate *L. monocytogenes* from the surface of products that have been re-contaminated between cooking and packaging. This procedure is currently practiced commercially by certain manufacturers throughout the world.

Post-cooking exposure and contamination

The cooking step for cooked meat products can be controlled in facilities operating with effectively designed and implemented HACCP plans, but preventing contamination of the cooked product during cooling and packaging is much more difficult. Contamination of the production environment and equipment, and transfer of *L. monocytogenes* during processes such as cooling, slicing and packaging is a significant cause of product contamination. A properly applied environmental sampling program for, e.g., *Listeria* spp., (see Chap. 10) can detect possible harborage sites/niches in the plant. Product design, cleaning and sanitation are among the activities needed to prevent contamination of the final product.

Composition of product – predictive models for growth

It is well recognized that *L. monocytogenes* can grow well on deli-meats that do not have growth inhibitors (McDonnell et al. 2013). Although compounds such as lactate and diacetate can suppress the growth of *L. monocytogenes*, the extent of inhibition can differ depending on factors such as storage temperature, type of deli meat, product pH, within and between lot variation in the concentrations of *Listeria* inhibitors, and the composition of product microbiota (Zhang et al. 2012). Predictive models for the growth of *L. monocytogenes* in RTE meats have been developed (Mejlholm et al. 2010) that accurately predict growth under a wide variety of conditions. For example, the Danish Meat Research Institute has developed a free online predictive modeling program that predicts the growth rate of *L. monocytogenes* in relation to seven variables (temperature, NaCl, pH, L-lactate, acetate, nitrite and percent CO_2 in the headspace) in freshly produced meat products (please see http://dmripredict.dk/Default.aspx).

Time and temperature in the supply chain
The growth of *L. monocytogenes* on deli-meats is significantly affected by the temperature and time of storage, which is under the control of the manufacturer, retailer or consumer at different times in the life of the product. Product and environmental conditions such as pH, water activity, temperature, and antimicrobials can be combined to provide conditions in which *L. monocytogenes* cannot grow a meat product, i.e., application of a multi-hurdle approach to controlling growth.

16.2.3 Factors Affecting Dose-Response

There are different dose-response models for healthy individuals and for high-risk populations such as pregnant women, the elderly, and immunosuppressed people. The actual risk varies widely between these populations. It has been estimated that various subpopulations may have a 20–1100-fold increased risk of acquiring listeriosis (Goulet and Marchetti 1996; FDA/FSIS 2001; Goulet et al. 2012).

Based on the observation that serovars 1/2a, 1/2b and 4b dominate among the strains isolated from human cases, whereas a wider range of serovars have been isolated from foods, it has been suggested that this is a reflection of their different potential for causing disease (FAO 2004). An analysis of the serovars of *L. monocytogenes* isolated from 1363 patients demonstrated that serovar 4b was the most common, being present in 64% of cases, whereas serovars 1/2a, 1/2b, and 1/2c were detected in 15%, 10%, and 4% of cases, respectively. Serovar 4b was more common in pregnancy-associated cases, and serovar 1/2b occurred more often in non-pregnant individuals with severe underlying disease (McLauchlin 1990; Doyle 2001). However, when tested in pregnant mice, infectivity of serovars1/2a and 1/2b did not differ significantly from 4b (Lammerding et al. 1992; Doyle 2001). Mutations in the gene *Internalin A* have been related to virulence differences among *L. monocytogenes* subtypes (Van Stelten et al. 2011).

Hazard characterization
Human listeriosis is now widely recognized as primarily a foodborne disease caused by the ingestion of *L. monocytogenes* in food. This also includes secondary transmission between a mother and her fetus or neonate. Despite its widespread presence in the environment, illness due to this organism occurs infrequently. The outcome of listeriosis can be severe with an estimated case-fatality rate of between 20 and 30% in the segments of the population most “at risk”, i.e., immunocompromised individuals, pregnant women, and the elderly. This spectrum of individuals is estimated to comprise 15–20% of the population (Buchanan et al. 1997), and is expected to increase, reflecting the trend in longer life expectancy and an overall aging population.

Host variability
Unlike the symptoms caused by many foodborne pathogens, diarrhea and other gastrointestinal symptoms are not common symptoms of listeriosis, although the patient may experience malaise and a mild fever. In several foodborne listeriosis outbreaks, patients have only exhibited these mild symptoms (Salamina et al. 1996; Dalton et al. 1997; Ryser and Buchanan 2013). However, the majority of reported cases have been the invasive type of listeriosis, symptoms of which can include meningitis, encephalitis and septicemia. In pregnant mothers, unrecognized and untreated listeriosis can lead to abortion of the fetus, stillbirth or premature delivery of a sick child. Serious sequelae such as mental retardation and hydrocephalus also have been reported following cases of neonatal listeriosis (Büla et al. 1995; Batz et al. 2013).

In risk assessments, the differences in host susceptibility can be calculated separately, as was done in the FAO/WHO risk assessment where an average figure of 40 times greater susceptibility was used to distinguish between the normal and high-risk groups. Goulet et al. (2012) reviewed cases of listeriosis reported in France from 2001 to 2008 and calculated risk ratios for various high-risk groups as

compared with normal individuals <65 years of age. For example, pregnant women were at 116-times greater risk. It should also be noted that although adults from 65–74 years of age were only 8-times at greater risk, individuals >74 years of age were at 20-times greater risk.

Strain variability
It is known that there are differences in virulence between different serotypes of *L. monocytogenes*, with serotype 4b generally been acknowledged as being the most virulent. However, even within the same serotype, differences in virulence can exist. Some of the strains that have been associated with reduced virulence have been linked with the production of truncated internalins (Van Stelten et al. 2011).

Dose-response
There are insufficient data from which to build a reliable dose–response model for *L. monocytogenes* either from experimental outbreak data, human volunteer feeding trials or animal experiment data (Ross 2009a–d). FDA and FSIS (2003) used an approach based on the use of a "dose–response scaling factor" to "correct" a mouse-derived model for the range of virulence to make it applicable to humans, but that model varies with every iteration of the risk assessment and is neither readily reproduced nor readily defined. FAO/WHO (2004) used the FDA and FSIS, 2003 study to infer a global dose–response model without the need for adjustment factors using essentially the same approach as Buchanan et al. (1997). This approach, also used by Ross et al. (2009a, b, c, d), extends the Buchanan et al. (1997), Lindqvist and Westöö (2000) and Chen et al. (2003) derivations of *L. monocytogenes* dose–response relationships, because it is based on 20 different ready-to-eat food commodities rather than one, and also considers the effect of growth of *L. monocytogenes* in the products between the time of "sampling" for the presence and concentration of *L. monocytogenes*, and the concentration at the time of consumption.

A significant vulnerability of the dose–response model used is that it relies on the validity of all assumptions and data used in the development of the FDA and FSIS (2003) *L. monocytogenes* risk assessment. If any of the assumptions or data were altered in such a way as to alter the estimated level of exposure, the resulting dose–response model would, by inference, be changed, as would any estimates of risk based on it. The data of Gombas et al. (2003) and Levine et al. (2001), for example, suggest that more recent estimates of the prevalence of processed meats contaminated with *L. monocytogenes* are lower by a factor of two or three than was apparently used in the FDA/FSIS risk assessment FDA/FSIS 2003. The FAO/WHO risk assessment (FAO/WHO 2004) discussed how the dose-response curve of the earlier risk assessment was dependent upon the underlying assumption of the maximum population density that *L. monocytogenes* could attain in a food product. A general change in the assumption of storage times or temperatures, particularly for higher risk products, could significantly affect this estimation process.

16.2.4 Risk Characterization

Risk Characterization
Risk characterization represents the integration of the hazard identification, hazard characterization, and exposure assessment determinations to obtain a risk estimate; providing a qualitative or quantitative estimate of the likelihood and severity of the adverse effects which could occur in a given population, including a description of the uncertainties associated with these estimates. These estimates can be assessed by comparison with independent epidemiological data that relate hazards to disease prevalence.

Deli-meats are consumed in large quantities throughout the world. The 2003 FDA/FSIS risk ranking model was developed to identify the relative risk of illness or death posed by RTE foods in 23 categories (FDA/FSIS 2003). This assessment indicated that deli meat posed the greatest public health

risk for listeriosis of all the RTE foods, i.e., according to the FDA/FSIS risk ranking model, roughly 80% of all deaths and cases were thought to be caused by deli-meats.

The consumption of deli-meat in the USA has been slightly increasing in the past decade. Since it is a high-ranking risk food that is often served to vulnerable populations, it is important to prevent foodborne listeriosis associated with the product. Cates et al. (2006) found that knowledge associated with *L. monocytogenes* is particularly low among seniors (≥ 60 years), which is of concern as they are a major risk group. Cates et al. (2006) also found that another risk group, pregnant women, were not aware of the risks of foodborne listeriosis and were not following prevention practices. In general, consumers tend to store their deli meats for longer than the recommended shelf life. There has been an increase in messaging to reach the vulnerable groups. For example, The Centers for Disease Control and Prevention (CDC), recommendations are that people aged 50 years or greater should reheat their cold meat before eating, to avoid listeriosis. For those over 50, and especially over 65, the recommendation is that meats such as hot-dogs, cold cuts, luncheon and deli meats should be heated to at least 165 °F(74 °C), which the CDC describes as "steaming hot" (FDA 2011).

According to the USA census in 2013, the population of the USA was around 315 million. From 2009 through 2013, the estimated incidence of listeriosis from the FoodNet system was 3.4, 2.8, 3.1, 2.6 and 2.6 cases per million per year, respectively (CDC 2012). Of the illnesses and deaths from *L. monocytogenes* from deli-meat consumption in the USA, approximately 83% were attributed to deli meat sliced and packaged at retail establishments, and 17% to prepackaged deli-meats. (Endrikat et al. 2010; FSIS 2010). The estimated mean number of deaths per year associated with prepackaged product was 34.1, and the estimated mean number of deaths per year associated with retail-sliced product was 166.9, with an estimated total annual number of deaths equal to 201. A total of 17% of the estimated per annum deaths (34.1/201.0 = 16.96%) were attributable to prepackaged product, while the remaining 83% (166.9/201.0 = 83.03%) were attributable to retail-sliced product (FSIS 2010).

The prevalence and level of *L. monocytogenes* in RTE meat and poultry deli-meats at retail establishments were determined using data from a study conducted by the NAFSS in which 6 of 3522 (0.17%) samples and 49 of 3518 (1.39%) samples tested positive for *L. monocytogenes* from prepackaged and retail-sliced deli meats, respectively. Fifty-seven samples were found to be positive for *L. monocytogenes* resulting in an overall prevalence rate of 0.76%. Based on this analysis, RTE meat and poultry products sliced at retail are approximately 4.9 times more risky on an annual basis than prepackaged product in terms of deaths from listeriosis. Retail-sliced products are associated with 83% of all *L. monocytogenes* deaths from deli meats. This percentage is largely unaffected by consumer storage time, product shelf life, or total number of *L. monocytogenes* associated deaths. Overall, there was no statistically significant difference in the prevalence of *L. monocytogenes* among the different deli meat types (FSIS 2010).

16.3 Risk Management

16.3.1 Principal Control Measures

Contamination of deli-meats primarily occurs after heat processing, i.e., post-processing contamination. Control of the post-processing environment is therefore critical in the control of *L. monocytogenes* in deli-meats. In addition, since most of the illnesses and deaths associated with deli-meats contaminated with *L. monocytogenes* are due to deli-meats sliced and packaged at retail, sanitation at retail, especially with regards to deli-meat slicers, display cases, and sinks is very important (Ford and Opper 2014). Shelf-life dating and control of the chill chain are important factors.

16.3.1.1 Primary Production

Controlling initial levels in raw materials
Proper handling of raw materials during storage and preparation to minimize an increase in numbers due to contamination or growth can be important. Controls at primary production can be important for certain foods, e.g., raw milk cheeses made from the milk of cows with listerial mastitis. For deli-meats, fermented sausage products excess levels of *L. monocytogenes* in the raw meat could exceed the ability of the subsequent fermentation to inactivate the pathogen.

16.3.1.2 Manufacturing

A flow diagram for deli-meats can be seen in Fig. 16.1. There can be considerable variation in raw materials, product formulation, cooking, and post-cooking handling processes. These variations may result in changes to the risk and the vulnerability of the product at each step of the production process. GHP and HACCP must be used to achieve the required level of control of microbiological hazards (FSO) for the particular product, its ingredients, formulation, method of manufacture, packaging, storage and distribution, and shelf-life. It is important to consider available control measures relevant to a particular product and the methods used in its manufacture. The following control measures for *L. monocytogenes* may be applicable.

Cooking
Cooking must be sufficient to eliminate *L. monocytogenes* in the center of a raw meat emulsion or injected whole muscle. Target reductions in the range of 5 to 6 logs should be appropriate.

Preventing recontamination between cooking and packaging
Many, if not all deli-meats are susceptible to recontamination between cooking and packaging. Minimizing recontamination between cooking and packaging by adopting GHP measures is required for most products.

L. monocytogenes is a very hardy organism and can be recovered from different locations in the plant environment depending on the level of control. In the absence of an effective control program, the organism can persist for prolonged periods in production environments. Thus, even if a performance objective is met for a cooked deli-meat, recontamination of processed product is a possibility unless proper attention has been paid to GHPs targeted towards the control of *L. monocytogenes*.

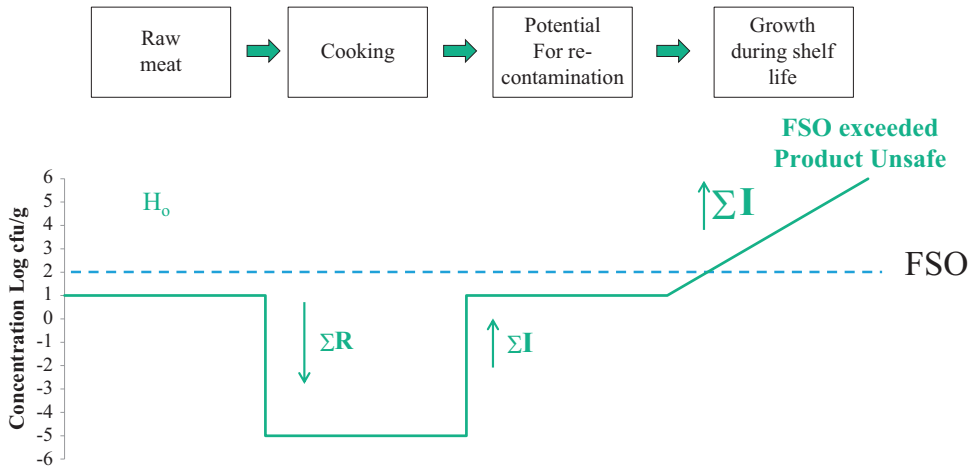

Fig. 16.1 Lack of control of *Listeria monocytogenes* in deli-meats where product supports growth, leading to an unsafe product

Certain measures that fall within the realm of GHPs have been found to be important for the control of *L. monocytogenes* in cooked meat and poultry operations. The measures include plant layout, equipment design, maintaining equipment, cleaning and sanitizing procedures that are specific to listeriae control, maintaining a clean and dry environment, the use of enclosed steam to sanitize equipment as a scheduled routine procedure, and low temperature storage (Tompkin et al. 1999; Rajic et al. 2007).

Two factors determine the effectiveness of a listeriae control program, i.e., routine environmental testing and the response to a positive finding. Without an environmental testing program, it can be difficult to assess control. Furthermore, in the event a positive product contact sample is detected, corrective actions should be initiated to eliminate the source of contamination, thereby minimizing the risk of product contamination. To verify control, plants should implement an environmental monitoring program for *L. monocytogenes* or an indicator such as *Listeria innocua or Listeria* spp. The program must be specific to each plant and should detail the areas to be sampled, the analytical method to be used, the frequency of sampling and the action to be taken when *Listeria* spp. are detected (Tompkin et al. 1992, 1999).

An effective monitoring program to assess control of the cooked product environment should consider the following strategies:

- preventing the establishment of *L. monocytogenes* in niches or other sites that can lead to contamination of RTE foods
- implementing a sampling program that can assess in a timely manner whether the RTE environment is under control
- responding to each positive product contact surface sample as rapidly and as effectively as possible
- verifying that the problem has been corrected, including providing data (e.g., tabulated, trends in graphical form) to facilitate short and longer term assessment of control

Experience in cooked meat and poultry operations indicate that a common source of contamination results from niches. These are sites within the cooked product environment wherein *L. monocytogenes* becomes established and multiplies. The sites are typically within equipment and are impossible to reach and clean with normal cleaning and sanitizing procedures. In fact, the processing environment typically appears visually clean and acceptable. The sites serve as a reservoir from which the pathogen is dispersed and contaminates product contact surfaces. Microbiological testing is necessary to detect the niche.

Response to a positive product contact sample (e.g., a sponge sample collected from a conveyor belt used in a cooked meat operation) is best when the following is considered. Assuming an effective control program is in place, the primary source of contamination is a niche. In general, contamination flows downstream through the process flow much like a river. It is important that the source (i.e., niche) be located. This can best be accomplished by creating a map of the cooked product rooms and the equipment layout (see Chap. 11). The results of samples collected from each piece of equipment should be recorded on the map, including both positive and negative results. The layout map should then be reviewed for patterns. Which sites that could have an impact safety of the product are more frequently positive? Where in the process flow do the first positives occur? It is important when seeking the source to analyze all the environmental samples separately and not as composites. In addition, sampling should be increased both in terms of location and frequency throughout the period of operation. Unfortunately, niches can rarely be detected unless the equipment is operating and product is being produced. While pursuing the source of contamination, consideration must be given to the possibility that a niche may not be involved (e.g., someone touching the floor or other unclean surface and returning to handling exposed product). When equipment has been identified as the source, the following steps can be effective. First, dismantling the equipment and collecting samples of suspicious

material as this is being done. Second, replace obvious defective parts (e.g., hollow rollers) and clean and sanitize the equipment as it is being re-assembled. If this procedure fails to eliminate the cause (or source) of contamination, it may be necessary to remove sensitive electronics, oil, and grease and heat the equipment. This can be accomplished by placing the equipment in an oven and heating to an internal of 71 °C with high humidity. Alternatively, the equipment can be shrouded with a tarp and steam can be injected into the space until the equipment reaches an internal temperature of 71 °C. Internal temperatures can be monitored with strategically placed thermocouples.

In cooked meat operations, the following are examples of identified sources of contamination:

- rubber gaskets around the doors and other openings to brine chill systems
- saturated insulation on pipes carrying refrigerant to the brine chill system
- peelers that remove artificial casings before packaging
- casing removal systems
- hollow rollers on conveyors
- on/off valves and buttons for various equipment
- within complex collating equipment
- hollow support rods on the frames of equipment
- deep in the bowels of cutting/slicing machines

In retail operations, for deli-meats, sources of contamination include cross-contamination from display cases to open deli-meats, utensils including knife racks, cutting boards, scales, employees and/or their gloves, deli preparation sinks, and, most importantly, meat slicers (Hoelzer et al. 2012).

Reducing levels in cooked product after packaging (in-pack pasteurization)

Applying processes for pasteurizing the product after packaging (i.e., in-pack pasteurization) is an approach that seeks to reduce any post-cooking contamination with *L. monocytogenes* to safe levels. Pasteurization through the application of heat or high pressure can have a significant effect in reducing risk (Ross et al. 2009a; Patterson et al. 2011; Juck et al. 2012). Cold pasteurization may be achieved through the application of high hydrostatic pressure, which can also extend the shelf-life of products (Hayman et al. 2004; Patterson et al. 2011; Juck et al. 2012). This method may not be suitable for some products or packaging systems.

There are also commercial preparations available whereby meat products are sprayed with a mixture of listeriae bacteriophages. For example, the bacteriophage preparation LISTEX™ has been approved by several regulatory agencies throughout the world including the USFDA, Health Canada and FSANZ. It is a processing aid which contains a mixture of phages characterized by their broad spectrum of activity against *L. monocytogenes*. A reduction of 1 to 3 logs has been reported in meat products (Listex 2014 http://micreosfoodsafety.com/en/listex-productdata.aspx), and a 1-log reduction was also observed in a study involving inoculated cat fish fillets (Soni et al. 2010).

Preventing growth through product formulation: Antimicrobial additives

In general, it can be assumed that deli-meats will be formulated with a high water activity and pH close to neutral, such that growth of *L. monocytogenes* is supported. Preventing the growth of *L. monocytogenes* in these products is a risk mitigation strategy that can be an alternative to in-pack pasteurization.

Numerous studies have demonstrated the value of antimicrobial additives such as sodium lactate/diacetate to extend the lag phase and reduce the growth rate of *L. monocytogenes*. (Hwang and Tamplin 2007; Pal et al. 2008; Stasiewicz et al. 2011). A risk assessment done by Pradhan et al. (2010) showed that reformulation of deli-meats with growth inhibitors was estimated to reduce human listeriosis deaths linked to ham and turkey by 2.8- and nine-fold, respectively. Furthermore, the use of starter cultures to control the growth of *L. monocytogenes* in cooked meat products is another tool that manufacturers can use. In fact, there are certain bacterial strains that have been already been approved for use on meat products, e.g., *Carnobacterium maltaromaticum* (FDA 2009).

In addition, validated models have been developed that predict the lag phase and growth rate of *L. monocytogenes* in meat and other products (Stopforth et al. 2010; Mejlholm et al. 2010; Purac, 2012; Danish Meat Institute 2015; Combase).

16.3.1.3 Distribution/Marketing

Control of time and temperature in the supply chain
Controlling the increase in numbers of *L. monocytogenes* that subsequently may occur in packaged product during storage and distribution is a function of time and temperature. The maximum time that product may be held is determined by labeling a date on the package (use-by, best before, sell-by, etc.) depending on labeling regulations within a country. The temperature of storage is also significant, because no storage temperature, short of freezing, will prevent the growth of *L. monocytogenes* and the organism will grow much more quickly in, e.g., deli-meats stored at 10 °C than at 4 °C. Furthermore, the microorganism becomes more resistant to multiple hurdles as the temperature of storage increases. Thus, the control of time and temperature is critical when product formulation is used to control the growth of *L. monocytogenes*.

Cross-contamination at retail
While a large volume of deli- meats are sold in vacuum or modified atmosphere packs (MAP), a significant proportion may also be sliced at retail, with products generally being consumed within a few days. GHPs must be applied at retail establishments, particularly the separation of products with a high and low risk of being contaminated with *L. monocytogenes* and the cleaning and sanitizing of slicing equipment. Cross-contamination at retail does occur and can be quite significant. (Pradhan et al. 2011; Hoelzer et al. 2012; Hammons and Oliver 2014).

16.3.1.4 Preparation/Consumption

Consumer storage
Consumers may store the product after purchase, thereby extending supply chain time and temperature issues; however, there may be less control over these factors in the hands of a consumer.

Heating prior to consumption
Deli-meats RTE, and consumers do not usually cook these products before consumption.

Advice to vulnerable populations
These could be achieved as control measures in food service operations or through education of consumers, particularly the more susceptible populations and their health care providers. Many countries have developed excellent material on how high-risk consumers can reduce their risk of acquiring foodborne listeriosis e.g., www.foodsafety.gov/poisoning/risk/, www.fda.gov/newsevents/newsroom/pressannouncements/ucm314077.htmwww.

16.3.2 Establishing an ALOP/Food Safety Objective

In most industrialized countries, the annual incidence of listeriosis is between 2 and 6 cases per million of the population (ECDC 2010; CDC 2011; Ryser and Buchanan 2013), figures that have not changed significantly in the last decade. In terms of food attribution, the number of cases of listeriosis

linked to deli-meat consumption has a high degree of uncertainty; however, there has been a significant decrease in the last 10 years, as more and more companies are using either growth inhibitors or high-pressure processing as additional control steps. The FDA/USDA risk assessment published in 2003 found that among 23 RTE foods, deli-meats posed the greatest risk of listeriosis, and was estimated on an annual basis to be responsible for 1600 cases of listeriosis and approximately 300 deaths. Ross et al. (2009a, b, c, d) developed a risk assessment model that predicted that processed meats could be responsible for up to 40% of cases of listeriosis in Australia. An example of a public health goal could be to reduce the number of cases attributable to the consumption of deli-meats by some value (e.g., 50% reduction).

Due to its widespread presence in the environment, eradication of *L. monocytogenes* from the food supply is impossible. There is general agreement that when the organism is ingested in low numbers, even susceptible individuals have little chance of developing listeriosis. A realistic FSO must therefore be established that would, as far as possible, recognize that it is not possible with current technology to eliminate contamination of foods with *L. monocytogenes*. When total prevention is not possible, measures must be put into place to control re-contamination and/or growth to an acceptable level. To achieve these goals, it is essential that GHP and HACCP programs that are specific to the control of *L. monocytogenes* be applied at all stages of manufacture, storage, transport and retail. In addition, research should continue to develop additional barriers to control the growth of *L. monocytogenes* in deli-meats.

It is currently recognized that on a daily basis, humans ingest *L. monocytogenes* in foods at low levels (e.g., 1 to 10 cfu/g) without becoming ill. In addition, most countries (EU, Canada, etc.) which have established action levels for *L. monocytogenes* of 100 cfu/g for low-risk products based, in part, on the fact that similar rates of foodborne listeriosis are observed in those countries as compared to countries which have a "zero tolerance" policy. This is also consistent with the estimated dose response curve for *L. monocytogenes* (FAO/WHO 2004) which includes an estimate of immunocompromised individuals, is reasonably conservative, and allows for the possibility of a single cell causing serious illness. Epidemiologic data indicate that foods involved in listeriosis outbreaks are those in which the organism has multiplied and in general have contained levels well in excess of 100 cfu/g (see Table 16.1). Thus, on the basis of epidemiologic and prevalence data, the following food safety objective (FSO) has been proposed:

The concentration of *L. monocytogenes* in cooked deli-meats should not exceed 100 cfu/g at the time of consumption.

This proposal is consistent with an earlier recommendation from ICMSF (ICMSF 1994), as well as with the latest Codex Alimentarius recommendations. It also is compatible with a conclusion of the FAO/WHO risk assessment for *L. monocytogenes* in ready-to-eat foods that a more strict tolerance of "not detected in 25 g" does not provide a higher level of protection (FAO/WHO 2004). In fact, the risk assessment demonstrated that the vast majority of cases of listeriosis result from the consumption of high numbers of *L. monocytogenes*, regardless of whether the standard is zero tolerance (0.04 cfu/g) or 100 CFU/g if growth of *L. monocytogenes* can be controlled throughout the shelf life of the product until consumed. FSO's can also be calculated from dose response curves (see Chap. 2).

16.3.3 Identifying and Establishing Performance Objectives

A performance objective is the maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption that provides or contributes to an FSO or ALOP, as applicable (see Chap. 3). Performance objectives are usually applied at steps where hazards can either be reduced or where hazards can increase.

The following sections provide an example of the calculation of a PO from the FSO. Several processes significantly affect the risk of listeriosis: cooking, post-pack pasteurization, and the addition of antimicrobial additives.

To arrive at a PO for the control measures needed to meet an FSO for cooked deli-meats, the equation from Chap. 3 can be used:

$$\text{Ho} - \Sigma R + \Sigma I \leq \text{FSO}$$

$$\text{Ho} - \Sigma R + \Sigma I \leq 2.0$$

where:

FSO = Food safety objective
Ho = Initial level of the hazard
ΣR = the total (cumulative) reduction of the hazard from processing, etc.
ΣI = the total (cumulative) increase of the hazard

FSO, Ho, ΣR, and ΣI are expressed in $\log_{10}$ units, ΣR is expressed as the number of log cycles of reduction achieved and ΣI is the log cycles of increase that occur as a result of both growth and recontamination.

The equation is then rearranged to solve for Ho.

$$\text{Ho} \leq \text{FSO} + \Sigma R - \Sigma I$$

Since the ΣR and ΣI apply only to the process(es) subsequent to the point at which the PO is defined, PO effectively is the Ho value for the process between the PO and the FSO.

$$\text{PO} \leq \text{FSO} + \Sigma R - \Sigma I$$

It is important to note that with the exception of the FSO, the values above are not constants, but have distributions which are assumed to be represented by the mean log concentration and its standard deviation. The examples below are calculated such that the POs represent values that would ensure that the FSO is met with 95% confidence.

16.3.3.1 Manufacturing

Recontamination following cooking

As already noted, any re-contamination event has the potential to lead to a situation where *L. monocytogenes* can multiply to reach high numbers during the subsequent distribution and storage, unless products have been formulated to prevent the growth of *L. monocytogenes*. High numbers may be consumed unless there is in-pack pasteurization (next section) or re-heating prior to consumption (unlikely).

Experience indicates that re-contamination after cooking, i.e., post-processing recontamination, is the most common reason for the presence of *L. monocytogenes* in deli-meats.

Dealing with recontamination may require an informed decision on the part of the manufacturer in terms of quantifying its contribution to the overall impact of ΣI. If the recontamination event takes place after cooking but prior to growth, the log increase of the recontamination would be added to the level of the pathogens after cooking. This would serve as the baseline value for subsequent increases due to growth. For example, if the level of *L. monocytogenes* was reduced to $\log_{10} = -3$ after cooking and then the meat was recontaminated to a level of 10 cfu/g which subsequently grew by 4 log cycles, the ΣI would be 1 + 4 = 5. However, if recontamination occurred after growth, then the calculation would be somewhat different in that the calculation would need to be done arithmetically and then converted to log values. For example, if we already have a $\log_{10}$ value of 3.0 due to growth of *L. monocytogenes* and a recontamination event adds an additional 100 cfu/g, then it would be appropriate to

convert to arithmetic values, add them, and then reconvert to log numbers, i.e. 1000 cfu/g + 100 cfu/g = 1100 cfu/g, which is log cfu/g = 3.04.

If we use the example of a deli-meat which has become re-contaminated after cooking at 10 cfu/g, has no risk mitigation steps (no re-heating, no antimicrobial additives) and supports a 5-log increase during shelf-life, then:

$$\begin{aligned}
\mathrm{PO}_{\text{after packing}} &\le \mathrm{FSO} + \Sigma R_{\text{re-heat}} - \Sigma I_{\text{after packing}} \\
\mathrm{PO}_{\text{after packing}} &\le 2 + 0 - \Sigma I_{\text{after packing}} \\
\mathrm{PO}_{\text{after packing}} &\le 2 + 0 - 5 \\
\mathrm{PO}_{\text{after packing}} &\le -3
\end{aligned}$$

$\mathrm{PO}_{\text{after cooking}} > \mathrm{FSO}$ and therefore the product is unsafe.

Reducing levels in cooked product after packaging (in-package pasteurization)
An in-package pasteurization treatment could be used to provide a final 4-log reduction.

Using the same example as above:

$$\begin{aligned}
\mathrm{PO}_{\text{after cooking}} &\le \mathrm{FSO} + \Sigma R_{\text{re-heat,pasteurization}} - \Sigma I_{\text{after packing}} \\
\mathrm{PO}_{\text{after cooking}} &\le 2 + 0 + 4 - \Sigma I_{\text{after packing}} \\
\mathrm{PO}_{\text{after cooking}} &\le 2 + 0 + 4 - 5 \\
\mathrm{PO}_{\text{after cooking}} &\le 1
\end{aligned}$$

$\mathrm{FSO} \le \mathrm{PO}_{\text{after cooking}}$ and the product is therefore safe.

It should be recognized that the parameters that are used in the equations above are actually point (deterministic) estimates, whereas in practice, they will have a distribution (stochastic) of values associated with them. If data exist for the variance associated with the different parameters, the underlying probability distributions can be established using an approach similar to that used when doing a risk assessment. As an illustration, the example above is shown again below, however, this time a stochastic approach is used.

In the example above, using a stochastic example, if the H_o is 1.0 log cfu/g with a SD of 0.8, the SD for ΣR (4 log reduction) is 0.5 and the SD for ΣI (5 log increase in growth) is 0.8, then the FSO would actually be exceeded 50% of the time. However, under the same conditions, if one wanted to have a much greater chance of meeting the FSO, then, e.g., by decreasing the H_o to −1 log cfu/g, the FSO would actually be met 95% of the time (Zwietering et al. 2010).

Some other examples of how one can use the PO to operationalize the microbiological criteria are shown in Figs. 16.2, 16.3 and 16.4. These figures demonstrate various scenarios whereby *L. monocytogenes* is either able or not able to grow on a deli-meat. Re-contamination as well as an additional terminal kill step, i.e., HPP, is also shown for illustration purposes.

16.3.3.2 Distribution

Time and temperature of storage:
Most, if not all deli-meats that do not contain growth inhibitors will allow some growth of *L. monocytogenes*. Codex Alimentarius (2007- annex II 2009) defines that *L. monocytogenes* will not grow in foods that have:

- a pH value below 4.4,
- an a_w value <0.92,
- a combination of factors (pH, a_w), e.g., a combination of a pH value <5.0 with an a_w value of <0.94.

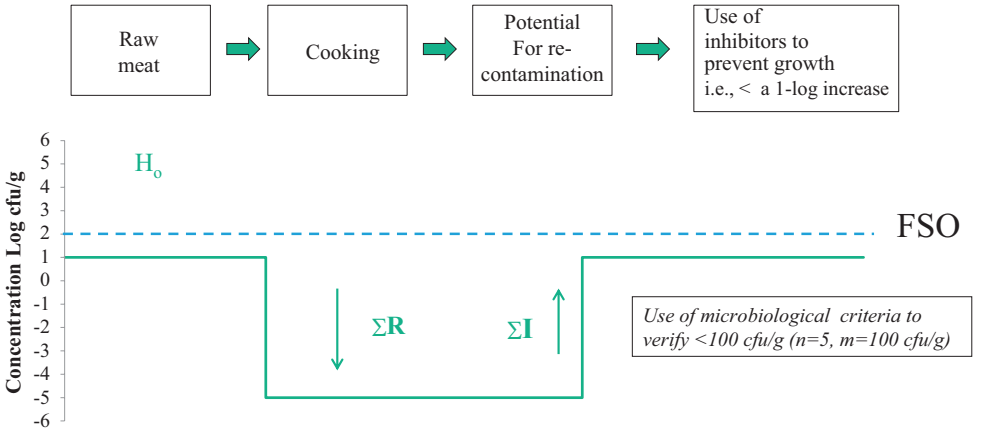

Fig. 16.2 Control of *Listeria monocytogenes* in deli-meats through the use of chemicals to inhibit growth

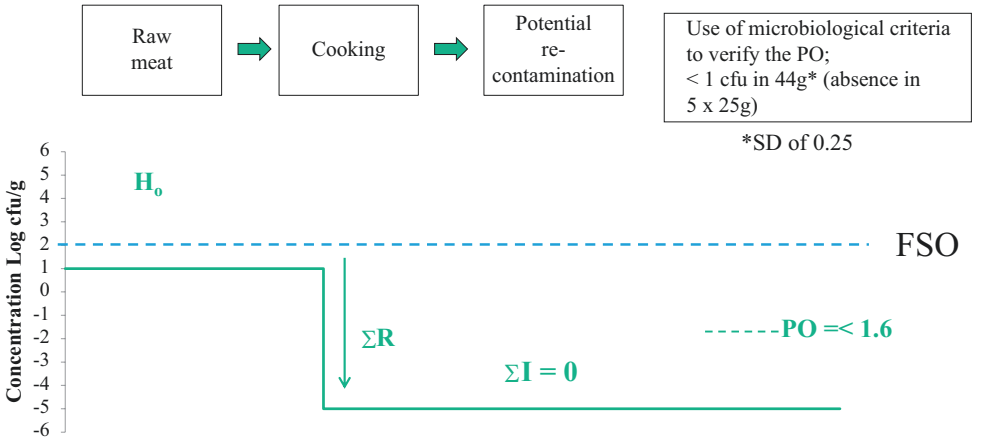

Fig. 16.3 Control of *Listeria monocytogenes* in deli-meats where product supports growth, through prevention of recontamination and verification using microbiological criteria

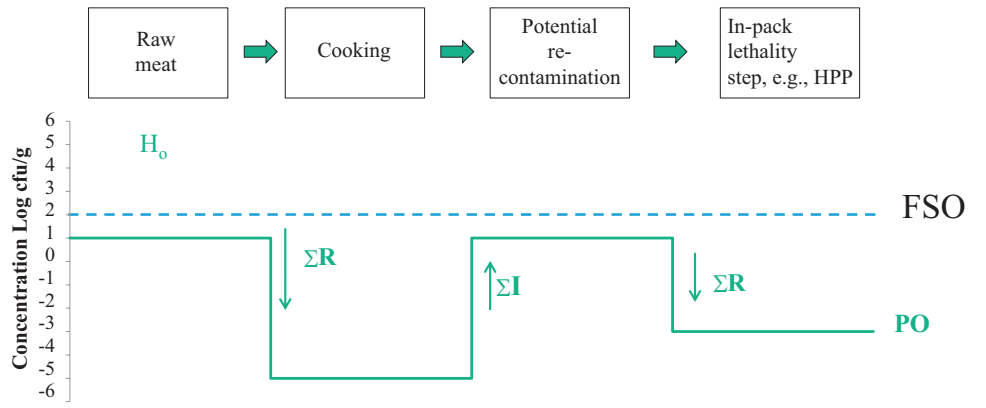

Fig. 16.4 Control of *Listeria monocytogenes* in deli-meats where product supports growth, is recontaminated after cooking, but receives an in-pack treatment

Inhibitors that can control the growth of *L. monocytogenes* can work synergistically with other extrinsic and intrinsic factors.

Demonstration that *L. monocytogenes* will not grow in a ready-to-eat food can be based upon, for example, food characteristics, the study of naturally-contaminated food, challenge tests, predictive modelling, information from the scientific literature and risk assessments, historic records or a combinations of these. Such studies must be appropriately designed to validate that *L. monocytogenes* will not grow in a food. Examples exist of how to perform challenge testing and conduct validation studies (Health Canada 2012c).

Predictive models can be very useful tools to consider the growth that may occur during the storage of the product prior to consumption. Several models have been developed to predict the growth of *L. monocytogenes* in processed foods, validated against a range of foods and compared with one another (Buchanan and Whiting 1996; Mejlholm et al. 2010; Danish Food Institute; Combase). Intrinsic factors such as pH and a_w at the values likely to be found in cooked deli-meats, provide good conditions for the growth of *L. monocytogenes*. A 1-log increase per week can be expected at 5 °C in many products. The addition of nitrite or smoke components (measured as phenol) would likely lengthen the lag phase and reduce the growth rate of *L. monocytogenes*. In addition, the presence of weak organic acids and their salts (e.g., potassium lactate, sodium acetate/diacetate) can also lengthen the lag phase and/or reduce the growth rate of *L. monocytogenes*.

The times and temperatures in the supply chain can vary widely. The time can be conveniently divided into: time in the manufacturer's warehouse, transport, retail display and consumer storage – all of which may have an associated time and temperature. The means by which the end of shelf-life is signaled to consumers is also an important consideration.

Given the foregoing discussion, growth in a deli-meat product that has few, or ineffective microbial inhibitors, could easily allow a 5-log increase or greater in numbers of *L. monocytogenes*. As previously stated, this value can be set deterministically, or a frequency distribution of potential growth could be used. In this case, using the example of a product which receives no re-heating prior to consumption, and a deterministic estimate of growth:

$$\begin{aligned}
\mathrm{PO}_{\text{after packing}} &\le \mathrm{FSO} + \Sigma R_{\text{re-heat}} - \Sigma I_{\text{after packing}} \\
\mathrm{PO}_{\text{after packing}} &\le 2 + 0 - \Sigma I_{\text{after packing}} \\
\mathrm{PO}_{\text{after packing}} &\le 2 + 0 - 5 \\
\mathrm{PO}_{\text{after packing}} &\le -3
\end{aligned}$$

If effective microbial inhibitors are used, growth can be prevented through the shelf-life of the product. In fact, the predictive model can be used to set a safe shelf-life, in which no growth or limited growth to <100 cfu/g occurs. Using the same example,

$$\begin{aligned}
\mathrm{PO}_{\text{after packing}} &\le 2 + 0 - 0 \\
\mathrm{PO}_{\text{after packing}} &\le 2
\end{aligned}$$

16.3.3.3 Preparation/Ingredients

Manufacturers of cooked meats should assume that *L. monocytogenes* will be present in all species and types of raw meat and poultry used in the preparation of deli-meats. This is evident from reports in the literature and results from surveys such as the USDA baseline studies for ground beef, ground turkey, and ground chicken (USDA 1995a, b). Data of this nature can be used to establish the initial concentration (H_O). Another option would be to generate similar data from product collected at the facility prior to cooking.

The USDA baseline studies reported the prevalence of *L. monocytogenes* in raw ground beef, chicken, and turkey to be in the range 18–35% when analyzing 25 g samples (ICMSF 2002). Further analysis of the positive samples showed that *L. monocytogenes* was present in low numbers. It can be concluded from the data that concentrations of 100 cfu/g or fewer would be expected under normal conditions. Consideration should be given to the multiplication of *L. monocytogenes* that might occur as raw meat and poultry is being stored and prepared for cooking.

To further place the possibility of multiplication during preparation into perspective, the data derived from Combase (http://modelling.combase.cc/ComBase_Predictor.aspx) predicts that it would take around 7 days for a ten-fold increase in numbers of *L. monocytogenes* (4 °C, 0.5% NaCl, pH 6.5). Longer times would be required in raw materials containing added salt. For example, the mechanically de-boned poultry meat used for manufacturing cooked meats typically contains added salt and sodium nitrite, and is chilled to well below 4 °C before shipping to the manufacturing plant.

16.3.4 Performance Criteria

A performance criterion is the required outcome of a step, or combination of steps, that contribute to ensure that the PO/FSO is achieved. In the case of deli-meats, the reduction is ensured by a heat-treatment, followed in some cases by a reduction as a result of a post-packaging high-pressure or hot water process treatment. Performance criteria set at 6D for the heat process and 3 to 4D for a high-pressure processing treatment would be realistic. In addition, post-package pasteurization of fully cooked meat products (weighing up to 9 kg) by water submersion (96 °C) for about 10 min should achieve a 2- to 4-log inactivation of *L. monocytogenes* on the product surface.

16.3.4.1 Process Criteria

Thermal Processes

Thermal processes are applied in the cooking process, and optionally, in post-packing pasteurization. Each manufacturer must determine the parameters for heating that will provide the desired product quality, cost, and, in this example, ensure the performance objective is met for cooked product. The parameters adopted by one manufacturer may differ from those adopted by others. This is due to differences in equipment, desired product quality, type of raw materials (e.g., beef, pork, chicken and/or turkey), type of casing (e.g., natural, artificial), method of applying smoke (e.g., natural, liquid), fat content and other factors. To meet the performance objective requires knowledge of the heat sensitivity of *L. monocytogenes*, particularly in the product being heated.

The thermal process can be validated by different means. One could be to conduct thermal inactivation studies in the laboratory using deli-meat emulsion material inoculated with a mixture of *L. monocytogenes* isolates from meat operations. Another method may be to perform in-house challenge experiments with non-pathogenic "surrogate" organisms such as *L. innocua*. Appropriate reference material that describe the factors that need to be considered should be consulted before conducting studies to validate a thermal process.

A third approach to validation is to review the published literature for data on thermal inactivation resistance. For example, numerous studies have been published that report thermal inactivation values for *L. monocytogenes* in various meat and poultry products (Farber 1989; Zaika et al. 1990; Mackey et al. 1990; Fain et al. 1991; ICMSF 1994; Farber and Peterkin 1999; Murphy et al. 2004).

The data summarized in Table 16.3 provide thermal inactivation values for *L. monocytogenes* in meat and poultry cooked to temperatures within the range of 60° to 68 °C.

A number of factors influence the rate of death, but from the data it can be concluded that a wide variety of different time/temperature combinations can be effective for inactivating *L. monocytogenes*

Table 16.3 Reported thermal inactivation values for *L. monocytogenes* in meat and poultry

Product	Temp (°C)	D-value (min.)	Reference
Beef steak	63.9	2.2	Gaze et al. (1989)
Chicken breast	63.9	1.6–1.8	
Ham – control culture	60	1.8	Carlier et al. (1996)
Ham – heat shocked culture	60	3.5	
Ham – "resistant" culture	60	1.0	
Cured sausage blend of 66% pork and 33% beef	63.9	3.3	Farber and Brown (1990)
Cured sausage blend of 66% pork and 33% beef	30 min at 47.8 °C then heat to 63.9 °C	4.2	
Cured sausage blend of 66% pork and 33% beef	60 min at 47.8 °C then heat to 63.9 °C	4.7	
Cured sausage blend of 66% pork and 33% beef	120 min at 47.8 °C then heat to 63.9 °C	8.0	
Meat slurry	68	0.23	Aguilar et al. (2011)
Chicken thigh/leg	60	2.04	Murphy et al. (2004)
	64	0.30	
Chicken skin	60	3.95	Murphy et al. (2004)
	64	0.41	
Sausage (pork)	60	7.3	Doyle et al. (2001)
Sausage (23% beef, 77% pork)	60	9.13	Doyle et al. (2001)
Beef	60	4.67	Juneja (2003)
Beef	65	0.75	Juneja (2003)
Beef	71.1	0.17	Juneja (2003)
Beef	73.9	0.04	Juneja (2003)

D value = time in minutes at a specified temperature for a one $\log_{10}$ (i.e., ten-fold) reduction in the number of *L. monocytogenes*

in a wide variety of foods. Such data can be used to arrive at alternative thermal processes for cooked meat products, and to consider the disposition of products in the event of a deviation.

An example of a default process criterion to achieve a 6 $\log_{10}$ reduction for in-pack pasteurization can be found in the draft Code of Practice developed by a Belgium-Dutch Chilled Meals Working Group, which recommends an internal heat treatment of 70 °C for 2 min for the elimination of *L. monocytogenes* in chilled, long-life pasteurized foods (Gaze et al. 1989; Lund and Notermans 1993). The advantage in using a performance objective rather than a default process criterion is that it allows flexibility in how the performance objective is achieved. This would facilitate the development of new technology that may offer novel ways to achieve the same end result.

High-pressure processing

High-Pressure Processing (HPP) consists of applying high pressure to a packaged food product via compressed water. Prior to pressurization, packaged meat and poultry products are loaded in a tray, which is then loaded into the pressure vessel. The vessel is sealed and then pressurized by injecting water into the vessel until a defined pressure specific for the food to be treated is reached. The packaged RTE products are thus immersed in the pressurized water and subjected to high hydrostatic pressure. HPP can be used as a post-processing, post-packaging intervention step for the control of *L. monocytogenes* in RTE meats and poultry, and in some cases to extend the shelf life of these products (Doona et al. 2012; Stollewerk et al. 2012).

Recently, Health Canada issued guidance on the use of HPP post-lethality treatment to reduce *L. monocytogenes* levels in RTE meats and poultry products. A letter of no objection was issued to the use of HPP at 87,000 psi/600 MPa for a minimum cycle time of 3 min to the maximum of treatment length of 27 min (Health Canada 2012a).

16.3.4.2 Product Criteria

Product criteria that are intended to prevent growth after packaging could be validated by performing challenge studies using surface-inoculated cooked meat to assess the likely increase (ΣI) of *L. monocytogenes* before the recommended use-by date. Challenge studies could involve, for example, using commercial product inoculated and packaged in a laboratory with a mixture of five isolates of *L. monocytogenes* stored at temperatures to which the product would be exposed during storage and distribution (Mellefont and Ross 2007; Health Canada 2012c). Many factors should be considered when conducting such studies. Predictive modeling also can provide an estimate of the pathogen's behavior and possible growth or inactivation (Mejlholm et al. 2010).

If product criteria (pH, a_w, concentration of weak acid preservatives, nitrite, smoke, etc.) are intended to prevent growth of *L. monocytogenes*, then attention needs to be given to the degree of safety in the formulation, and the precision of process control required to ensure that all lots of production conform in all aspects to the requirements. Sensitivity analysis and process control approaches may be useful to determine the critical limits needed to prevent growth and set product criteria.

16.3.4.3 Shelf-Life Limits

Shelf-life limits can be defined in terms of product acceptability to consumers or in restricting the opportunities for *L. monocytogenes* to grow. It is likely that without in-pack pasteurization, product will become unsafe before it becomes organoleptically unacceptable. If antimicrobials are used to prevent the growth of *L. monocytogenes*, the length of the lag phase may need to be balanced with the organoleptic acceptability of the product.

A uniform procedure has not been developed to validate code-dating practices to ensure the safety of perishable foods with extended shelf life (e.g., cooked meat or poultry products). As discussed in Chap. 3, a number of factors must be considered when validating the effectiveness of one or more steps along the food chain. The following factors should be considered when performing a validation study for code dating:

- physiological state of the inoculum (e.g., 24 h broth culture)
- method of inoculation (surface)
- inoculum level (per package or per g)
- source and number of strains
- temperature(s) of storage
- natural competitive flora (commercial v pilot plant product)
- product formulation (fermentable carbohydrate and rate of acid production)

16.3.5 Acceptance Criteria for Final Product

16.3.5.1 Organoleptic

Each type of deli-meat has particular and characteristic organoleptic qualities which will depend on its composition and certain of these parameters are used for their release.

16.3.5.2 Chemical and Physical

Cooked meat and poultry should comply with relevant national regulations or the guidance laid down in the Codex Alimentarius Commission. These requirements are, on the one hand related to the nutrient composition and, on the other hand, to criteria for chemical contaminants.

16.3.5.3 Microbiological

Routine sampling of deli-meats for *L. monocytogenes* is not recommended. However, if the routine application of GHPs and HACCP is in question, sampling for *L. monocytogenes* would be appropriate. The sampling plans for *L. monocytogenes* should follow the ones recommended by Codex Alimentarius (CAC 2007). More specifically, when evidence indicates a potential for contamination with *L. monocytogenes*, e.g., positive food-contact surfaces results or the effectiveness of corrective actions has yet to be verified, sampling of deli-meats should be considered. When it is known that the product has been post-pack pasteurized, sampling final product would provide no added value and is not recommended. Likewise, for those plants that are using a validated kill step for cooking and where an effective environmental sampling program documents that the risk of re-contamination is being controlled, there is little value in testing end product. The reason is that a comprehensive management system can maintain frequencies of contamination to less than 0.5%. Under these circumstances, the frequency of defective units is too low for detection with any practical sampling plan (see Chaps. 6, 7 and 8).

Where product is crossing international borders and nothing is known about the product or the manufacturing process, end product testing may be appropriate. For deli-meats supporting growth, the sampling plan recommended would be n = 5 × 25 g, with a c = 0 and m = absence in 25 g (< 0.04 cfu/g). Assuming a log normal distribution, this sampling plan would provide 95% confidence that a lot of food containing a geometric mean concentration of 0.023 cfu/g and an analytical standard deviation of 0.25 log cfu/g would be detected and rejected if any of the five samples are positive for *L. monocytogenes*. Such a lot may consist of 55% of the 25 g samples being negative and up to 45% of the 25 g samples being positive. In addition, 0.5% of this lot could harbor concentrations of *L. monocytogenes* > 0.1 cfu/g.

16.4 Relating Risk Management Metrics to the Stringency of a Food Safety System

16.4.1 HACCP Programs

The heat-treatment applied during processing is considered as a CCP and needs to be managed as such. In addition, if HPP is used, this could also be considered as a CCP. Critical limits could vary depending on the type of inactivation process applied.

Considering the stringency of the hygiene control measures required to control listeriae in a deli-meat processing facility, other measures such as zoning and the sampling of food contact surfaces would be considered as prerequisite programs and could be managed as such.

With a properly designed and implemented HACCP plan, there would be no benefit to testing the cooked product for further validation of the process or to verify each lot has been cooked correctly. If an event occurs requiring such a need, then a sampling plan of n = 5, c = 0 with an m = absence in 25 g (< 0.04 cfu/g), could be used.

16.4.2 GHP Programs

Certain aspects of GHPs must be controlled to minimize recontamination of cooked products and have been discussed in this chapter. Effective control of *L. monocytogenes* demands diligent and consistent adherence to GMPs because of its prevalence in the environment, ease of spread, and ability to flourish in the RTE processing environment. These GMPs constitute the minimum requirements for the control of *L. monocytogenes* and historically were considered adequate for that purpose, but it is now clear that in many cases, more control is needed in many plants. Only establishments with strict adherence to the GMPs, a superior sanitation program, which includes regular deep cleaning of equipment, and a robust environmental testing program verifying effective control, should contemplate relying solely on those measures.

Other establishments should implement additional antimicrobial interventions to inhibit growth of *L. monocytogenes* in their products or to eliminate the organism altogether. The aim of conducting an environmental monitoring program is to verify, for example, that a manufacturer has successfully identified and controlled niches as well as harbourage sites for *L. monocytogenes* in the food operation, and to verify that sanitation programs have been appropriately designed and implemented to control contamination by *L. monocytogenes*. Sampling both food contact surfaces (FCS) and non-food contact surfaces (NFCS) enables a more complete understanding of the effectiveness of the *Listeria* control program within the plant. Testing for *Listeria* spp. and reacting to positive results as if they reflected the presence of *L. monocytogenes*, provides a more sensitive and cost-effective control program than would testing for *L. monocytogenes* alone.

16.4.3 Regulatory Requirements and Criteria

Microbiological criteria for *L. monocytogenes* have been included in the Guidelines on the Application of General Principles of Food Hygiene to the Control of *L. monocytogenes* in Foods (CAC 2007c). In addition, a number of regulatory authorities throughout the world have also established regulatory requirements and/or criteria (EC 2005; Health Canada 2011, 2012b).

The Codex microbiological criteria apply from the end of manufacture until the point of retail sale. These criteria differ according to whether the product is capable of supporting the growth of *L. monocytogenes*. In both cases, a 2-class plan is used; for products supporting growth, a qualitative method for the absence of *L. monocytogenes* in 5 × 25 g is expected, whereas for products in which growth will not occur, a quantitative standard of 100 cfu/g applies.

The qualitative method assumes the application of the ISO 11290–1 method. Assuming a log-normal distribution, this sampling plan would provide 95% confidence that a lot of food containing a geometric mean concentration of 0.023 cfu/g and an analytical standard deviation of 0.25 log cfu/g would be detected and rejected if any of the five samples are positive for *L. monocytogenes*. Such a lot may consist of 55% of the 25 g samples being negative and up to 45% of the 25 g samples being positive; 0.5% of this lot could contain concentrations above 0.1 cfu/g.

The quantitative method assumes the application of the ISO 11290–2 method. Assuming a log-normal distribution, this sampling plan would provide 95% confidence that a lot of food containing a geometric mean concentration of 93.3 cfu/g and an analytical standard deviation of 0.25 log cfu/g would be detected and rejected based on any of the five samples exceeding 100 cfu/g of *L. monocytogenes*. Such a lot may consist of 55% of the samples being below 100 cfu/g and up to 45% of the samples being above 100 cfu/g, whereas 0.002% of all the samples from this lot could be above 1000 cfu/g. If there are concerns that too many of the samples are clustered just below the 100 cfu/g limit, an appropriately designed three-class plan could be substituted.

The typical actions to be taken where there is a failure to meet the criteria would be to

- Prevent the affected lot from being released for human consumption
- Recall the product if it has been released for human consumption
- Determine and correct the root cause of the failure

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Chapter 17
Enterohemorrhagic *Escherichia coli* on Fresh-Cut Leafy Vegetables

17.1 Introduction

This chapter is concerned with *Escherichia coli* O157:H7 in fresh-cut, ready-to-eat (RTE) leafy green vegetables, sometimes simply referred to as "leafy greens". The information follows the principles outlined in earlier chapters. The unique severity of *E. coli* O157:H7, and other enterohemorrhagic *E. coli* (EHEC), potentially present on RTE foods such as "leafy greens" warrants special consideration. Food safety risk management of RTE leafy vegetables products requires a holistic, or "systems" approach involving Good Agricultural Practices (GAPs), Good Manufacturing Practices (GMPs) and Good Hygienic Practices (GHP) in processing, minimization of growth during distribution and testing for validation and verification of control measures.

In this chapter, an example will be developed that explores the merits of microbiological testing, hygiene controls during packing, and temperature control during distribution as potential control measures to enhance the safety of RTE leafy vegetables. The example draws primarily on data from U.S., but the methods adopted and principles demonstrated are generally applicable to analysis of risk management strategies for leafy vegetables globally.

RTE leafy vegetables are considered an important part of a healthy diet; increased consumption of fruit and vegetables has been promoted by governments for improvement of public health (Suslow 1997; NHMRC 2003; FAO/WHO 2008b; USDA/DHHS 2010). RTE leafy vegetables include lettuce, chicory, spinach, cabbages and other brassicas (FAO/WHO 2008b). RTE "fresh-cut" produce, including bagged RTE leafy vegetable products and salad mixes offer convenience for consumers and, for these reasons, the fresh-cut industry has experienced strong growth over the past 10 years both due to retail consumers and food service. Global production of RTE leafy vegetables has been steadily increasing since the early 1960s, with a sharp increase in production from the early 1990s to 2013 that resulted in more than a doubling of production (FAOSTAT 2016) of lettuce and chicory, and nearly ten-fold increase in spinach production such that spinach production globally now matches lettuce and chicory with ~ 25 million tons produced annually in each category. In 2013, the major producers of lettuce and chicory were China (~68% of global production) and U.S. (~18% of global production), with India, Spain and Italy collectively contributing ~14% of global production. Production of lettuce and chicory in U.S. has steadily declined since 2005 (FAOSTAT 2016), while production of spinach has increased, though spinach production in U.S. is currently only ~0.4 million tons per year. In the U.S., from 1985 to 2013, per capita consumption of lettuce leaf products and Romaine quadrupled, with average per capita consumption being ~5 kg/year (USDA-ERS 2014).

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Foodborne infection outbreaks, including those from RTE leafy vegetables are increasingly reported internationally (NACMCF 1998; FAO/WHO 2008b; Lynch et al. 2009; Bell and Kyriakides 2009; Taban and Halkman 2011; CDC 2015; Spooner 2016; FSANZ 2016), leading to growing national and international concern. In response, an international expert consultation was convened by FAO/WHO (2008b) to provide scientific support to the Codex Alimentarius Commission on risk management for specific produce commodities. Those deliberations concluded that control of pathogens on RTE leafy vegetable products was of highest concern among all produce categories based on:

- frequency and severity of disease
- size and scope of production
- diversity and complexity of the production chain/industry
- potential for amplification of foodborne pathogens through the food chain
- extent of international trade and economic impact, and
- potential for control.

Anderson et al. (2011) reached an analogous conclusion, i.e., pathogenic *E. coli* in RTE leafy vegetables represent the highest food safety risk from produce in U.S., in terms of effect on public health, though not necessarily numbers of cases. EFSA (2013) concluded that while RTE leafy vegetables are a significant source of foodborne illness due to various pathogens, *Salmonella enterica* is most frequently implicated as the etiological agent. These conclusions are not, however, mutually exclusive and relate to the greater frequency of salmonellosis cases versus the greater severity of EHEC infections.

The microbiological safety of RTE leafy vegetables is problematic, particularly because the product is grown outside and can be subject to direct, sporadic fecal contamination from birds and animals. Contaminated agricultural water used for irrigation or foliar sprays, incompletely composted manure, and farm personnel are other potential sources of contamination of RTE leafy vegetable products, as is wind-blown contamination from distant sources of manure (Jahne et al. 2016). The product is relatively delicate and processing options to inactivate or remove pathogens are limited, e.g., prolonged heating is not a viable option. Currently there is no widely available and acceptable technology that can guarantee a high level of inactivation of EHECs or other pathogens on RTE leafy vegetables without compromising product quality or consumer acceptance of the product. Growth of EHECs can occur on the product (discussed below) under typical storage conditions and the product is consumed without cooking by the consumer. These circumstances suggest that management of the microbiological safety of RTE leafy vegetables requires a multi-faceted approach, addressing minimization of contamination, maximizing inactivation or removal of EHECs during processing, minimizing recontamination or cross-contamination during processing, and minimizing growth between production and consumption.

17.2 Risk Evaluation

17.2.1 Published Risk Assessments/Risk Profiles

Published risk assessments of EHECs on leafy vegetables include Franz et al. (2010), Tromp et al. (2010) Ottosen et al. (2011), Danyluk and Schaffner (2011), Pielaat et al. (2014), and Pang et al. (2017). The complementary risk assessments of Franz et al. (2010) and Tromp et al. (2010) focused only on the risk of EHECs on salad in salad bars, and did not consider factors influencing consumer risk along the full farm-to-fork chain. Pielaat et al. (2014) considered risks from several pathogens, including *E. coli* O157 in the Dutch production chain of mixed salads, concluding that risks from

E. coli O157 were orders of magnitude lower than from *Salmonella*, or *Campylobacter*. They commented that among the main sources of uncertainty in their model was the lack of decontamination data (e.g., the effectiveness of produce washing and disinfection during processing), a limitation that is addressed in this chapter.

Ottoson et al. (2011) focused their interest on management of pre-harvest steps, including quality of irrigation water and holding times after the last irrigation and before harvest but noted that rinsing lettuce in cold tap water for 15 s, prior to consumption, could reduce risk of EHEC infection six-fold. Danyluk and Schaffner (2011) considered all aspects of the farm-to-fork chain, to provide (comparative) risk estimates for pathogenic *E. coli* in RTE leafy greens. That assessment centered on the circumstances of the 2006 spinach outbreak in U.S., but was also intended to identify available data and also data gaps, but with less focus on elucidation of optimal risk management options, taking into account effectiveness and feasibility. Pang et al. (2017) also presented a farm-to-table risk assessment that examined the influence of different interventions on the risk from *E. coli* O157:H7 in RTE leafy vegetables.

17.2.2 Hazard Identification

Historically, fresh produce had not been considered a significant source of foodborne exposure to EHEC in the U.S. but, since the first outbreaks were reported in 1991, produce has remained a prominent vehicle of outbreaks in U.S. (Rangel et al. 2005; CDC 2015), most notably a 2006 outbreak associated with spinach (Todd et al. 2007; CDC 2012a). Batz et al. (2011a, 2012) evaluated the risk to U.S. consumers from a variety of foodborne pathogen-product combinations, including EHECs in produce. The risk from EHECs in produce was ranked 41st among the combinations considered by cost to society and loss of life quality of the victims. By way of comparison, the analyses suggested that *E. coli* in beef products ranked 21st. Batz et al. (2011b, 2012) identified 28 produce-related outbreaks, involving 1564 cases in U.S. from 1998–2008, due specifically to *E. coli* O157:H7. In the same study *E. coli* O157:H7 was estimated to have caused ~64,000 cases per year from all foods, while other EHECs (see Sect. 17.2.3) were estimated to have caused ~113,000 cases per year in U.S. from all foods. Of those estimated cases of *E. coli* O157:H7 infection, ~35,000 (55%) were considered to be related to beef products and ~11,500 (18%) to produce.

CDC (2015) identified 24 outbreaks related to *E. coli* in RTE leafy vegetables[1] in U.S. from 1998 to 2008. The number of reported cases was 950, similar to the reported number of cases attributed to beef products (namely 36 EHEC outbreaks involving 924 cases linked to beef products in the same period). In that period, there were 323 outbreaks from all foods, involving 8974 cases. Each of RTE leafy vegetables and beef products represented approximately 10% of all reported *Escherichia* infections that could be attributed to foods in U.S. during that period. EHECs in produce continue to cause outbreaks in the U.S.: CDC (2015) reported 11 further multistate outbreaks during 2013 and 2014 related to *E. coli* on RTE leafy vegetables.

[1]The CDC Foodborne Outbreak Online Database (http://wwwn.cdc.gov/foodborneoutbreaks/) was searched in late 2015 for outbreaks related to: green leaf lettuce, iceberg lettuce, iceberg lettuce (unspecified), leaf lettuce, leaf lettuce (unspecified), leafy green, lettuce, lettuce based salads, lettuce, prepackaged, lettuce (unspecified), mesclun mix, mesclun mix and spinach, mesclun mix salad, mesclun mix (unspecified), prepackaged leafy greens, romaine lettuce, shredded lettuce, spinach, spinach salad, spinach (unspecified).

17.2.3 Hazard Characterization

Escherichia coli O157:H7 is one of the serotypes associated with the EHEC pathotype based on the disease syndrome signs and symptoms, and virulence determinants. In differentiating and classifying *E. coli*, specific lipopolysaccharides on the surface of the outer membrane ('O' antigens) and proteins associated with flagella ('H' antigens) are distinguished. Hence *E. coli* O157:H7 strains are distinguished by having the cell surface antigen 157, and the flagellar antigen 7. While these antigenic properties do not necessarily mean the strain is pathogenic, many strains that are O157 and H7 positive do cause severe human illness due to presence of virulence genes. Other serotypes of EHEC are now recognized as equally important causes of foodborne human illness. For example, in U.S. testing for EHECs has been expanded from strain O157:H7 only to now include six other EHEC strains; serotypes O26, O45, O103, O111, O121 and O145 (USDA FSIS 2011).

Escherichia coli O157:H7 infection can result in moderate to severe disease or death, with most deaths occurring in children under 5 years of age and in the elderly (Tarr 1994). Three major syndromes of EHEC infections are recognized and include:

(i) hemorrhagic colitis: characterized by grossly bloody diarrhea, severe abdominal pain, vomiting, but no fever;
(ii) hemolytic uremic syndrome (HUS): characterized by a prodrome of bloody diarrhea, and with acute nephropathy, seizures, coma, and death; and
(iii) thrombotic thrombocytopenic purpura which is similar to HUS but also characterized by fever and central nervous system disorder (ICMSF 1996).

Scallan et al. (2011) estimated that, while there are ~3700 cases of EHEC infections reported per year in U.S., under-diagnosis may mean that the true number of cases (*i.e.*, including those not reported) may be as high as 63,000, consistent with the estimates of Batz et al. (2012).

The proportion of cases specifically attributable to RTE leafy vegetables can be estimated from the numbers of cases due to produce reported by Batz et al. (2012) and those due to RTE leafy vegetables estimated above, *i.e.*, 950/1564 cases = 61%. The estimate of Batz et al. (2012) was for *E. coli* O157:H7 specifically, while estimates from CDC (2015) are for all *E. coli* infections. However, the estimates presented above suggest that 36% of foodborne *E. coli* infections are due to *E. coli* O157:H7. Accordingly, the total number of EHEC infections from RTE leafy vegetables per year in U.S. is estimated as 18,200.

17.2.4 Dose Response Considerations

There are insufficient data to enable development of a reliable dose-response[2] relationship for the probability of infection/illness from EHECs. Note that in this case the response is the probability of illness (P_{ill}). Cassin et al. (1998), in their assessment of the public health risk from EHECs in hamburgers in North American culture, used a dose-response relationship based on data from three published human feeding studies (DuPont et al. 1969; DuPont et al. 1972; Levine et al. 1973) of *Shigella dysenteriae* and

[2] Strictly speaking, the term 'dose-response' in relation to infections is a misnomer because the consequences of infection are largely independent of the dose ingested. Although there are some reports of disease severity being affected by the dose ingested (e.g., Mintz et al. 1994) dose-response models in the microbial food-safety literature are for probability of infection, or probability of illness upon infection and should, more correctly be called 'dose-probability of illness' models. For simplicity, however, we use the term 'dose-response model' to indicate the relationship between EHEC dose ingested and probability of infection.

S. flexneri as a surrogate for probability of illness from *E. coli* O157:H7 upon ingestion. It is worth noting that *S. dysenteriae* is capable of producing Shiga toxin and is a recognized cause of HUS. In those three studies the subjects were healthy male volunteers, and the cells were administered in 30–45 ml milk, on an empty stomach, or after a dose of bicarbonate to neutralize gastric acidity. Their model extended the model of Crockett et al. (1996), based on the same data.

It should be noted, also, that there is variability in human susceptibility to infection and also variability in strain virulence. Accordingly, the probability of infection varies (Fig. 17.1) and can be characterized as a range of probabilities of infection. The response shown in Fig. 17.1a is described by a Beta-Binomial distribution. The ID_{50} (i.e., the dose level that results in 50% of a population becoming infected) can be used to compare the relative infectivity of pathogens. The ID_{50} dose that causes illness can vary between strains of a pathogen, according to the health of a consumer, the type of meal consumed etc. The ID_{50} can be regarded as a mid-point of the range of infectious doses from greatest to least likelihood of infection/illness.

Strachan et al. (2005) compared a range of dose-response functions fitted to outbreak data for EHECs and observed that the bounds of the outbreak models encompassed the Crockett et al. (1996) model. Parameter values of the Beta-Poisson models presented by Cassin et al. (1998) and the 'best fit' and 'median' models of Strachan et al. (2005) were used to reproduce and directly compare those dose response models (Fig. 17.1). From the Cassin et al. (1998) model, the ID_{50} for *Shigella* (as a proxy for EHEC) infections is ~2900 cells (ID_{50} = 3.46 log cells). Cassin et al. (1998) reported a standard deviation of 0.9 log cells (i.e., giving a 95% confidence interval of 46 to 182,000 cells). The Strachan et al. (2005) 'best fit' model based on EHEC outbreak data predicts an ID_{50} of ~200,000 cells, while their 'median' model was used to reduce the influence of apparently anomalous data. The Strachan et al. (2005) 'median' predicts an ID_{50} of 300 cells, and is more similar to the Cassin et al. (1998) model than the Strachan et al. (2005) 'best fit' model.

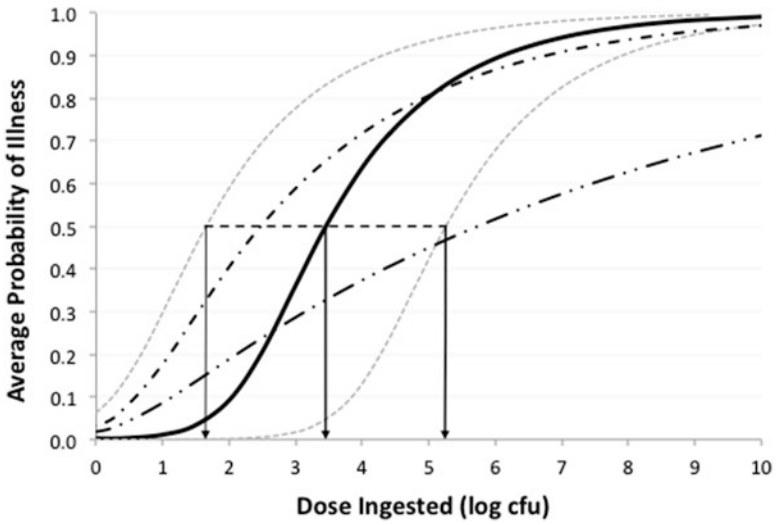

Fig. 17.1 Beta-Binomial dose–response model derived by Cassin et al. (1998) for *Shigella* spp. as a surrogate for EHECs (solid bold line), and showing the variability in the probability of illness *vs.* ingested dose of cells (dotted lines indicates the 95%-ile upper and lower confidence intervals on the response). Dose-$P_{illness}$ models of Strachan et al. (2005) based on outbreak data for EHECs and showing best fit (¨ _ ¨) and median (· _ ·) models together with confidence intervals for the exact beta-Poisson with beta-binomial likelihood

It is unclear which dose-response model is most plausible. FAO/WHO (2011) conducted an analysis of currently available dose response models for EHECs, including the Strachan et al. (2005) model. From that analysis the Cassin et al. (1998) model was shown to:

- provide a good representation of the average predictions of those various models, at least as a first approximation;
- give more conservative estimates than most of the other models; and
- encompass the confidence intervals of those other models.

Accordingly, the Cassin et al. (1998) model will be used in the current example, and has also received tacit endorsement from Ottoson et al. (2011) and Danyluk and Schaffner (2011). The Cassin et al. (1998) model infers an ID_{50} of ~2900 cells. As noted above, the data for that dose-response relationship was derived from volunteer studies, with the inoculum administered in milk. For the purposes of this study, it will be assumed that the dose-P_{ill} relationship is unaffected by food type.

17.2.5 Exposure Assessment

17.2.5.1 Pre-harvest Contamination

The microorganisms typically associated with RTE leafy vegetables are those associated with primary production. Normally, human enteric pathogens such as EHECs are not among the normal microbiota of RTE leafy vegetables but can arise from contamination of the primary production environment with microorganisms of human or animal origin. These can include birds, rodents, and domestic and wild animal populations if they are not excluded from production areas. Agricultural water can also become contaminated with fecal matter from humans or animals and contaminate RTE leafy vegetable crops during irrigation and other uses. Raw or inadequately composted manures can also transmit enteric pathogens to the soil, where they can persist for many months depending on temperature and soil moisture conditions. Contaminated soils can also contaminate crops with enteric pathogens via splashing during rain or irrigation, or during harvest.

Accordingly, because there is very limited potential to eliminate contamination (see Sects. 17.2.4.2 and 17.3.1.2), it is essential to take steps to prevent pre-harvest contamination of the product with feces of animals such as fencing to exclude larger animals, removal of habitats within the production regions to discourage smaller animals, devices to scare away birds, evaluation of agricultural water quality and hygiene, and confirmation that biological soil amendments have been fully processed to achieve a safe compost. The risks that flooding could introduce enteric pathogens from nearby sites, or risks from windborne contamination from animal feces in nearby locations (e.g., dairies, feedlots, etc.), should also be considered. Workers can also be a source of contaminants if toilet and personal hygiene facilities on the farm are inadequate. Harvest equipment should also be kept clean and free from contamination. More extensive advice on identification and prevention of potential sources of enteric contamination of leafy vegetables is presented, in ICMSF Book 8 (ICMSF, 2011, Chap. 12), CAC (2003a, Annex III) and LGMA (2013).

17.2.5.2 Hygiene Control and Cross-Contamination During Processing.

As discussed above, the primary means of safety assurance in RTE leafy vegetables is prevention of contamination, rather than subsequent removal of contamination. This is because the product is relatively delicate and cannot withstand heat treatments sufficient to eliminate microbial hazards without severely reducing the fresh characteristics of the product.

EHEC growth on the product is possible if the temperature is high enough (e.g., ~≥10 °C) and, accordingly, rapid cooling of leafy vegetables after harvest will minimize the potential for *E. coli* O157:H7 growth, while also preserving product quality and maximizing shelf life (see also Sect. 17.2.4.3).

RTE leafy vegetables are delicate and normally moved through processing *via* flumes. This includes steps to remove surface contamination and to apply disinfectants. The efficacy of disinfection is generally low because of the contact time possible (approximately 1 min) and because the flume water has to be kept cool to protect the product and maintain its potential shelf life. As such, the times and temperatures applied are not conducive to disinfectant efficacy. Longer treatment times or higher sanitizer levels can damage the product and reduce shelf life. Accordingly, the use of sanitizers is mainly to inactivate hazardous organisms in the flume water to prevent cross-cross contamination to uncontaminated leaves.

17.2.5.3 Predictive Models for EHEC Growth

The main source of change in EHEC concentration in leafy vegetables after processing is due to growth of EHEC in the product. Various studies, reviewed in Olaimat and Holley (2012), have presented data on growth of EHECs on leafy salad vegetables. Whipps et al. (2008) reported that the fate of EHECs on leafy vegetables is affected by nutrient availability, UV radiation, toxic compounds released by the plant, and competition from other microorganisms. Humidity and temperature have a strong effect on growth rate or inactivation rate (Ross et al. 2008). Delaquis et al. (2007) and McKellar and Delaquis (2011), however, reviewed the fate of EHECs in leafy vegetables and concluded that the most dominant influence on growth rate is temperature. Delaquis et al. (2007) further noted that growth is nutrient limited, and therefore slower, in comparison to predictions of most existing predictive models for EHECs, which are usually developed for nutrient rich foods.

Both Delaquis et al. (2007) and Danyluk and Schaffner (2011) collated growth rate data for EHECs on leafy vegetables from published studies. Those data show that growth does not occur below ~7 to 8 °C, as concluded by ICMSF (1996) and Shaw et al. (1971). From those reports typical growth rates at 10 °C are estimated at ~0.12 log cfu/day, and at 20 °C as ~0.7 log cfu/day. Franz et al. (2010) estimated EHEC growth in distribution chains in food service as ~0.06 log cfu over 24 h but observed average temperatures of approximately 5 to 6 °C and a maximum up to only 9 °C, generally below the limits for *E. coli* growth. Luo et al. (2010) inoculated *E. coli* O157:H7 onto Iceberg and Romaine lettuce and incubated them for 3 days at 12 °C. More than 2 logs of growth was noted, with further growth upon continued storage. Luo et al. (2010) further reported that although there was "a significant decline in visual quality of lettuce held at 12°C, the quality of this lettuce was still fully acceptable when *E. coli* O157:H7 growth reached a statistically significant level". However, very high levels of *E. coli* were inoculated onto the product and not all trials showed more than 2 log growth. It is possible that with more realistic contamination levels suppression of growth by spoilage microbiota may have be observed.

17.2.5.4 Time, Temperature and Potential for EHEC Growth in the Supply Chain

Ideally, the supply chain for RTE leafy produce would be below 4 °C so as to maximize the shelf life of the products. For fresh-cut lettuce held at 0 °C, a shelf life of up to 21 days can be achieved, but at temperatures of 5 °C, the expected shelf life is 7–10 days. At ambient temperatures the shelf life is as short as two days. Spinach has a shorter shelf life of only 14 days at 0 °C. Given the long supply chains for RTE leafy vegetables, refrigerated temperature control is essential for product quality. Several studies (FAO 2004; USFDA 2010) have found that temperatures of transport and retail display of RTE leafy vegetables are often above 10 °C, and thus would potentially permit proliferation of *E. coli*. Potential for EHEC growth during the shelf life and distribution of RTE leafy vegetables is considered in greater detail in Sects. 17.2.4.4 and 17.3.1.3.

Ideally, growth over the distribution and shelf life of the product would be based on actual data for time and temperatures experienced by the product in the distribution chain and consumer's home, so as to be able to integrate those data with growth rate models to predict growth between production and consumption. Two studies are available that have addressed these calculations. Danyluk and Schaffner (2011) used time and temperature data from a variety of published surveys of retail and home refrigeration temperatures and generated a predictive model for EHEC growth rate, also from a number of published studies on growth of EHECs on lettuce and leafy vegetables. From the predictive model, and time and temperature data, they used simulation modeling approaches to generate a distribution of 'amounts of growth' (Fig. 17.2a). A second study (Pérez Rodríguez, F., *pers. comm.,* 2011) also involved simulation based on measured data (Fig. 17.2b).

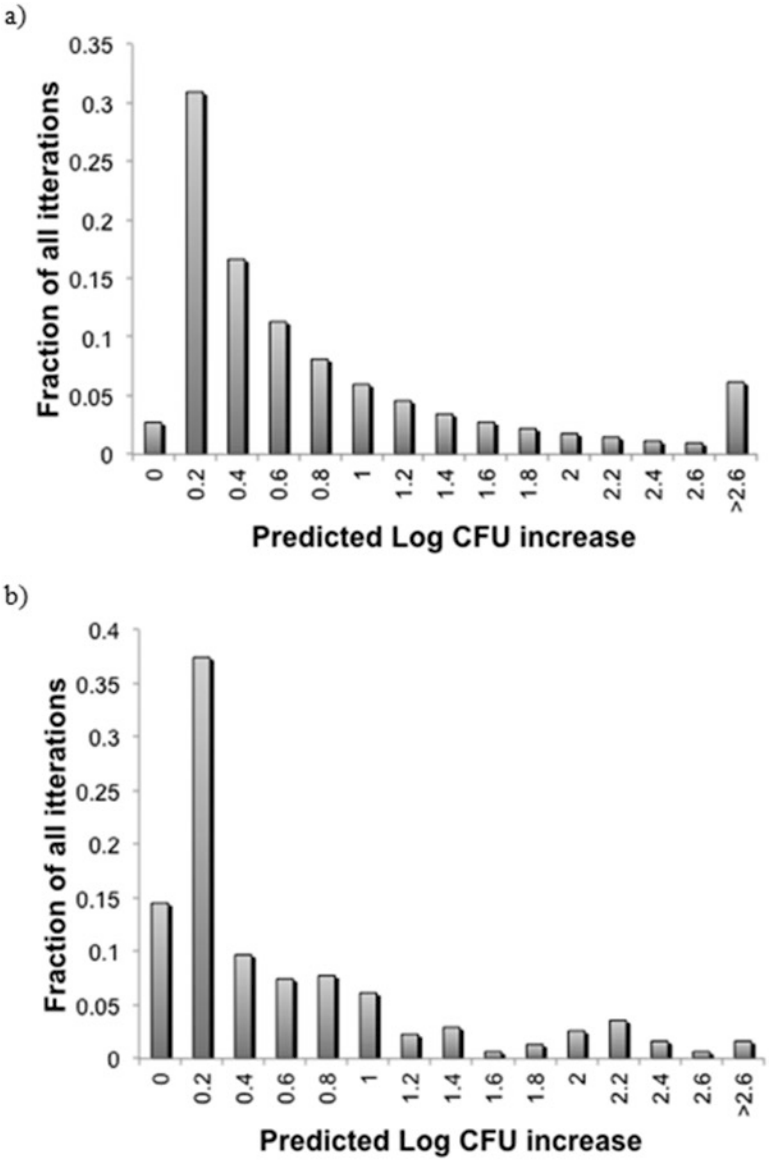

Fig. 17.2 Distribution of predicted log cfu increases on leafy green salad vegetables between processing and consumption generated by (**a**) Danyluk and Schaffner (2011) and (**b**) Pérez Rodríguez, F. (2011, *pers. comm.*) (**a** Reproduced, with permission, from Danyluk and Schaffner 2011)

There is strong similarity in the distribution of potential EHEC growth in both the Danyluk and Schaffner (2011) and Perez studies, with the mode of predicted growth in both being ~0.2 log cfu. The weighted means of predicted growth in those studies is ~0.6 log cfu, with the 95th percentile being ~2 log cfu. Koseki and Isobe (2005) recorded actual temperatures on Iceberg lettuce through distribution chains in Japan. They then simulated these temperature histories in the laboratory and monitored the growth of *E. coli* O157:H7 on Iceberg lettuce under these conditions. For three temperature histories, observed growth ranged between 0.8 and 1.0 log cfu between processing and the end of retail display, with no lag time observed. Conversely, as noted earlier (Sect. 17.2.4), Franz et al. (2010) estimated EHEC growth in distribution chains to food service as only ~0.06 log cfu.

17.2.6 Risk Characterization

Taking into account the above factors it is possible to develop a theoretical quantitative risk assessment model for the risk of EHEC infections resulting from consumption of RTE leafy vegetables. However, epidemiological data and estimates are available that provide a much more direct means of estimating that risk. In this situation, the value of a farm-to-fork risk assessment model lies more in the development and evaluation of alternative risk management options. This process will be demonstrated in Sect. 17.4.

From the epidemiological and consumption data presented, it can be estimated that approximately 18,200 infections per year in U.S. may be attributable to RTE leafy vegetable products. The population of U.S. in 2015 was ~322 million people (USCB, 2016). Total consumption of leafy salad vegetables in U.S. is currently ~5 kg per person (USDA-ERS 2014), similar to levels since 2010. Based on survey data, Hoelzer et al (2012) reported that ~45% of U.S. citizens consume fresh (i.e., uncooked) leafy salad vegetables, including spinach. A typical serving size is one cup, or ~85 g. Thus, it can be estimated that 18.9 billion servings are responsible for 18,200 EHEC infections. This risk translates to approximately one in 1.04 million servings (18.9 billion servings/18,200 cases).

17.3 Risk Management

17.3.1 Principal Control Measures

17.3.1.1 Primary Production and Processing

Existing control measures applied to primary production and processing of RTE leafy vegetables were described above in Sects. 17.2.5.1 and 17.2.5.2, respectively.

17.3.1.2 Distribution/Marketing

There is virtually no opportunity for additional contamination of bagged RTE leafy vegetables products after processing until the time of opening of the package by the consumer or food service operator. Consequently, the only reliable management option relevant to this section is temperature control. Keeping temperatures below 7 °C should prevent growth of EHECs in the product and prevent increased risk. Implementation of temperature control is discussed in Sect. 17.3.4.1.

17.3.1.3 Preparation/Consumption

Consumer Storage

Consumers may store the product after purchase, which is an extension of the time and temperature issues in the supply chain.

Washing Prior to Consumption

The additional washing of bagged RTE leafy vegetables by consumers is not likely to enhance safety. The risk of cross contamination from food handlers and food contact surfaces used during washing may outweigh any safety benefit that further washing may confer. Palumbo et al. (2007) recommended that leafy vegetables salads in sealed bags labeled "washed" or "ready-to-eat", produced in a facility inspected by a regulatory authority and operated under GHPs, do not need additional washing at the time of use unless specifically directed on the label.

17.3.2 Establishing an ALOP/FSO

A Food Safety Objective (ICMSF 2002) translates a public health goal into a measurable metric, i.e., a specified maximum frequency and/or concentration of a [microbiological] hazard in a food at the time of consumption, which is deemed to provide an appropriate level of health protection (see Chaps. 2 and 3). It is problematic to define a tolerable level of risk: ideally there would be 'zero risk' from food but, for many minimally processed ready-to-eat foods, currently this is not technologically feasible. Accordingly, some jurisdictions propose incremental reductions in risk as Food Safety Objectives.

The US Office of Disease Prevention and Health Promotion (ODPHP 2014) has set a health objective for the U.S. to reduce the 2006–2008 incidence of infections caused by *E. coli* O157:H7 of 1.2 cases/100,000 population to 0.6 cases/100,000, i.e., a reduction of 50% by 2020. For the purposes of this illustrative study we apply this same public health goal to leafy vegetables in U.S. To translate this aim into a Food Safety Objective, however, requires that the observed incidence of EHEC infections from produce be translated into risk per serving of leafy vegetables. In effect, this aim specifies an ALOP as no more than one illness per 2.08 million servings of leafy salad vegetables, i.e., 50% of the current rate.

To provide useful guidance to industry and regulators, this objective needs to be translated into a measurable quantity, e.g., a maximum frequency and/or concentration of a hazard in a food at the time of consumption. From the Cassin et al. (1998) dose-response model, a single EHEC cell would be expected to have a ~1 in 860 chance of causing illness in an 'average' consumer. To make this level clearer, if every serving of RTE leafy vegetables contained only one EHEC cell, we could expect ~1160 cases per million servings. Our target, however, is one case per 2.08 million servings (or 0.48 cases per million servings). Accordingly, the frequency of contamination must be lower than 1 EHEC cell per serving and must, instead, be reduced to $\leq$ 1 EHEC cell per ~2420 servings.

Assuming a typical serving of 85 g, our FSO requires that no more than one EHEC cell is present per 205 kg (205,000 g) of leafy salad vegetables at the point of consumption. Typical pack sizes of leafy green vegetables range from ~115–680 g. For convenience in the examples presented here, we assume that a package of RTE salad vegetables is 340 g, (equivalent to four servings): the corresponding contamination rate per bag is 1 EHEC cell per 604 bags.

To provide advice to industry about Performance Objectives and Process Criteria we use the 'ICSMF Equation' (Eq. 3.1):

$$H_o - \Sigma R + \Sigma I \leq FSO$$

and substituting into it the FSO established above:

$$H_o - \Sigma R + \Sigma I \leq 1/205{,}000 EHEC\ cells/\text{g} \tag{17.1a}$$

and, converting into more familiar units, i.e. log cfu

$$H_o - \Sigma R + \Sigma I \leq -5.31 \log \text{cfu/g} \tag{17.1b}$$

i.e., from the desired FSO the average contamination level in leafy vegetables at the point of consumption must be ≤ −5.31 log cfu/g. Section 17.3.5 considers the feasibility of testing product for conformity with this specification at the point of consumption.

17.3.3 Identifying and Establishing Performance Objectives

An aim of the ICMSF equation is to be provide guidance to food processors on hazard levels that should not be exceeded in their finished product so that, allowing for changes between processing and consumption, the product will still satisfy the Food Safety Objective (see Chaps. 2 and 3). This level is, effectively, the Performance Objective for the processor. Performance Objectives are targets by which food processors and others in the supply chain can chose and define processing control measures ('Performance Criteria) that are sufficient to meet the Food Safety Objectives, given the microbiological status of the ingredients they use. In the case of bagged RTE leafy vegetable products, the only ingredients are the vegetable leaves themselves. Apart from growth of EHECs on the product during distribution and storage, there are no points in the post-processing chain at which POs would usefully be specified, because there are no other critical processes where control is needed or can be applied.

Given the potential for growth, estimated to be 0.6 log cfu on average (Sect. 17.3.1.3), the hazard level at the completion of processing should be lower than the FSO. An average 0.6 log cfu increase equates to a four-fold increase in EHEC numbers between the end of processing and consumption. The proposed FSO translates to one EHEC cell or less per 194 bags of 340 g but, since growth of EHECs can only occur in bags that were contaminated at the end of processing, it is inferred that bags that are contaminated will have, on average, four EHEC cells in them at the time of consumption. From current understanding of dose-response relationships for infectious organisms (FAO/WHO 2010), at these low EHEC levels the probability of illness will be directly proportional to the number of cells. Accordingly, to maintain the FSO, tolerance for contaminated bags at the end of processing must be reduced by a factor of four to account for the average potential for growth. To satisfy the FSO no more than one 340 g bag in 777 may contain EHECs, i.e., a contamination level ≤ 1 EHEC/26.4 kg product, or ≤ −5.91 log cfu/g. This level of contamination is the Performance Objective (PO) for the product at the completion of processing.

17.3.3.1 Distribution/Marketing

In the calculations presented above an average of the status quo of temperatures in supply chains of leafy vegetables in U.S. has been assumed. Based on that status quo, and the PO derived for processors, the corresponding Performance Objective for distribution/marketing of the product should be such that the increase in contamination levels in the product does not exceed 0.6 log cfu, including the expected holding time and conditions of the consumer/food service segment. In practice, however, because the supply chain includes many steps and organizations, it would be impractical to set a

PO. Hence none is proposed. Nonetheless, it would be technologically feasible to set a PO that 'no increase in EHEC levels in product during distribution and marketing is permissible', relying on real-time temperature monitoring to assess the PO. This will be discussed further in Sect. 17.4.

17.3.3.2 Manufacturing/Port of Entry

Given the high perishability of these products and their short shelf life and short supply chain, the Performance Objective for Port of Entry inspection should be the same as for processor of the product. Similarly, because there is no further manufacturing involving these products, the same Performance Objective as for processing would be most practical and defensible.

17.3.3.3 Primary Production

An estimate of H_0 is needed to place the PO derived in Sect. 17.3.2 into context, i.e., if current on-farm management practices are sufficient to reliably achieve the PO, no processing to reduce EHEC levels would be required. Alternatively, if EHEC levels on raw materials are higher than the PO, additional processing for inactivation or removal of EHECs will be required.

Here we consider the levels of EHECs found on commercial products after harvest but before processing, and also the potential for inactivation or removal of EHECs during processing based on commercial data. Laboratory evaluations of efficacy of various currently used processing aids, and those proposed, are considered in Sect. 17.3.4.1.

As noted above, H_0 can be estimated from leafy vegetable testing data. Data, in confidence, were obtained from a number of leafy vegetable growers/processors. Numbers of test results in the databases were typically in the order of thousands, or even hundreds of thousands. The data describe contamination frequency both before and after processing. As illustrated in Sect. 17.3.5, the statistics of sampling will not allow a reliable detection of very low frequencies of contamination in a single lot of product. However, when tests are accumulated over a series of production batches, they provide validation of control measures of the production system (Zwietering et al. 2010). By analyzing the data across multiple production lots and consideration of the sampling methods, estimates of typical frequencies (and, from that, typical levels of contamination) can be estimated.

For one producer/processor, from 184,000 tests over a 1–2 year period, 290 were positive for EHECs or *E. coli* O157:H7 prior to processing, i.e., the prevalence is estimated at 0.16%. Conversely, from 90,400 samples after processing, 17 were positive for EHEC or *E. coli* O157:H7, i.e., a prevalence of 0.019%, and indicating an approximately 10-fold reduction in prevalence during processing. Based on advice received from the processor the test results are based on a 750 g sample (300 leaves). Thus, a crude estimate of the average contamination level in incoming product is 1 cell per 476 kg (−5.68 log cfu/g) prior to processing, or 1 cell per 3991 kg (−6.60 log cfu/g) after processing. A second, less intensive, sampling scheme is also employed by the processor involving analytical units of 60 leaves, or 150 g. If that less stringent scheme was employed, the average contamination prior to processing from the prevalence of positive samples is estimated at −4.98 log cfu/g and −5.90 log cfu/g after processing.

Other data obtained from USDA (2011b) provide a general indication of farm hygiene across all producers. There were 34 detections by PCR of EHECs in 4664 samples of bagged spinach, and bagged conventional or organic lettuce (0.73%), while only 0.4% were positive on culture. The detection rate in spinach was considerably higher than in either type of lettuce. Samples were taken after processing, and sample size was 450 g. From this we can infer an average contamination level of 1 cell per 113 kg (−5.05 log cfu/g) in the packed product.

17.3.4 Performance Criteria

17.3.4.1 Process Criteria

Processing

Chemical sanitizers are routinely included in flume water and wash water during commercial processing of fresh-cut leafy vegetables to minimize cross-contamination from the water (Davidson et al. 2013). EHEC are most likely to attach to stomata, irregularities on intact surfaces, cut surfaces, or cracks on the external surfaces of leafy vegetables (Gil et al. 2009; Parish et al. 2003; Sapers 2001). Washing of leafy vegetables remains important for removing soil and debris, decreasing the microbial load, improving quality and appearance, and enhancing product shelf life and safety (Herdt and Feng 2009).

Olaimat and Holley (2012) reviewed the efficacy of sanitizers currently used in commercial flume washing and found that most treatments rely on chlorine, which can achieve 1–3 log cfu reductions. Peroxyacetic acid efficacy ranges from 0.5 to 4 log cfu reductions, and depends at least partially on the product type. Inactivation was generally higher on smooth skinned fruits (e.g., apples and tomatoes) than on leafy vegetable products. Other chemical sanitizers achieve similar levels of inactivation.

Other technologies, while not widely used in industry, show potential to further control pathogens on leafy vegetables. Cold atmospheric plasma has been reported to inactivate >2 log cfu observed on lettuce. Irradiation at levels of ≤ 1 kGy is reported to reduce contamination with vegetative pathogens on lettuce by 2 to 4 log cfu. Other unpublished results (Niemira, *pers. comm.*, 2015) and reviews of published studies reinforce these results (Table 17.1).

It is apparent that the Processing Criteria will be dependent on the microbiological quality of the product at harvest, a function of pre-harvest interventions, and the potential for growth during distribution/marketing. These interrelationships will be explored in Sect. 17.4.2.

Distribution/Marketing

Lower temperatures extend the shelf life and preserve the quality of leafy vegetables. Recommended storage temperatures to achieve the desired shelf life were discussed in Sect. 17.2.4.3. If products were reliably stored at optimal temperatures for quality maintenance, no growth of EHECs would be possible. As such, specification of a maximum holding temperature during distribution/marketing could assist in risk minimization. Real time data logging technologies are now available and cost effective for many products. The use of temperature data could be enhanced by interpreting temperature data using a predictive microbiology model to establish more flexible Performance Criteria based on temperature, thereby allowing some tolerance for insignificant deviations above the lower temperature limit for EHEC growth (~7–8 °C, see Sect. 17.2.4).

Table 17.1 Input values (log cfu/g) for existing leafy vegetable products in U.S

Product	H_o	ΣR	ΣI
'High' risk	−5.68	0.92	0.6
'Normal' risk	−4.98	0.92	0.6
At retail	−5.05	n/a	0.5[a]

[a]Assumed value based on the assumption of the shortest period of time before the product becomes available for purchase at retail would be 3 days after processing and that shelf life after processing is 14 days

17.3.4.2 Product Criteria

From the above it can be seen that the efficacy of post-processing control influences the product criterion at the completion of processing. These interrelationships will be explored in Sect. 17.4.2. Relevant acceptance criteria, and the feasibility of monitoring them, are considered in Sect. 17.3.5. Instead, testing to evaluate control of the process will be more effective.

17.3.4.3 Shelf Life Limits

Fresh leafy vegetables are highly perishable and, during the shelf life of the product, only limited growth of EHEC would be expected before quality deterioration due to growth of other microorganisms on the product. As such, shelf life limitation is not considered a useful means of control of the risk of EHEC in these products.

17.3.5 Acceptance Criteria

17.3.5.1 Microbiological

The probability of detecting a faulty unit amongst a batch of units depends on the proportion of faulty units and the number of samples taken. The probability of detection can be calculated from the binomial distribution, often expressed as:

$$P_a = (1-p)^n \tag{17.2}$$

where p is the proportion of units in the batch that exceed the criterion for acceptability, and n is the number of sample units from the batch that must be tested and shown *not* to exceed that criterion to ensure that P_a, which is the probability that the sampling plan would *not* detect a batch with greater than the acceptable frequency of defective units, is acceptably low. Typically, P_a is selected to be 0.05, i.e., to give 95% confidence that the batch is acceptable. In fact, when testing is destructive, and samples are not returned to the batch after testing, the 'hypergeometric distribution' is the correct equation for calculating the probability of detection. When the number of samples taken is small relative the size of the batch, however, the binomial distribution provides almost identical results (Ross et al. 2011).

When testing for pathogens, we require a high level of confidence that if the batch does not conform to the specification, it would be detected so that the batch could be discarded. To satisfy the FSO, and assuming that a unit of RTE leafy vegetables, is a 340 g bag (4 servings), we aim to detect any batch with greater than 1/194 (0.0052) defective units, where any bag that contains even 1 cell is considered defective. From Eq. 17.2, and setting $P_a = 0.05$, ~570 analytical units must be tested and found to be free of EHEC (where an analytical unit is the entire contents of a 340 g bag) to accept the production batch and to demonstrate that it satisfies the FSO. If the analytical unit was 25 g, ~7750 tests would need to be performed to achieve 95% confidence that the batch satisfied the FSO.

Testing product at the point of consumption is not feasible and, as illustrated, requires impractically large amounts of the product. Instead, we need to translate the Food Safety Objective into a Performance Objective that applies at the point of processing. To do so, however, we need to estimate the change in hazard levels between the time of processing and the time of consumption.

The binomial distribution function (Eq. 17.2) can be used to assess the reliability of a testing regime. As an example, one producer tests 300 leaves (750 g) per batch of product that they consider to be 'high risk', including spinach and Romaine. The reasons for these designations are not clear, but probably relates to the frequency of outbreaks associated with those types of leafy vegetable. Zhou et al. (2009) also observed that *E. coli* are more difficult to remove from the underside of spinach leaves, presumably due to the leaf underside ultrastructure. A batch is ~182 kg, and a spinach leaf weighs approximately 2.5 g. The leaves are composited and enriched and the enrichment tested for the presence of EHECs. Assuming that the test methodology is perfect, i.e., that the method will detect a single EHEC if present in the 750 g composite sample, the maximum sensitivity of the test protocol can be evaluated. Again, adopting a 95% confidence level, we can evaluate Eq. 17.2, as follows:

$$P_a = (1-p)^n$$

$$0.05 = (1-p)^{300}$$

Solving for p, we find that p = 0.00994, i.e., only if more than one in ~100 leaves are contaminated, will testing 300 leaves enable that contamination to be detected in 95% of cases. In other words, the method will detect with 95% reliability whether there is more than one EHEC per 250 g. From this we infer the sensitivity limit of the testing regime is −2.39 log cfu/g, significantly less sensitive than required to directly assess achievement of the PO (≤ −5.91 log cfu/g). This level of testing (i.e., 300 leaves per batch) is used only for "high-risk" products: for other product types, fewer leaves per batch are sampled and composited. For 'lower risk' products a regime involving composites of 60 leaves (150 g) is used. Using the same approach, the sampling plan will only detect (at 95% confidence) batches in which the contamination level ≥ −1.71 log cfu/g (1 cell per 51 g). The evaluation of the reliable limit of detection of sampling plans is useful to assess whether product testing can provide effective risk management to achieve the FSO. Whether such plans do achieve this will depend on the levels of inactivation, or removal, of contaminants during subsequent processing steps. This is considered in Sect. 17.4.

17.3.5.2 Chemical and Physical

The presence of EHEC cannot be assessed by chemical or physical determinations and thus meaningful chemical or physical criteria cannot be established. Regular evaluation of sanitizer levels in flume and wash water is useful to ensure the efficacy of the process.

17.3.5.3 Organoleptic

The presence of EHEC in fresh cut leafy vegetables cannot be assessed by organoleptic evaluation and thus meaningful organoleptic criteria cannot be established.

17.3.5.4 Other

No other criteria are considered useful for product or process evaluation.

17.4 Risk Characterization and Risk Management

Risk characterization aims to synthesize data and knowledge about processes and events that contribute to risk and their relative contributions to the overall risk. From the understanding developed, we aim to find optimal strategies to minimize the risks.

17.4.1 Deterministic Approaches

The data presented above allows evaluation of how closely the current production, processing and distribution of leafy vegetables in the U.S. match the proposed FSO. We can use Eq. 17.1a and substitute values for H_0, ΣR and ΣI that were derived above to estimate the average concentration of EHECs at the time of consumption. Table 17.2 presents estimates of values that were derived.

Substituting the above values into Eq. 3.1:

$$H_o - \Sigma R + \Sigma I = -5.68 - 0.92 + 0.6$$
$$= -6.00 \log \text{cfu/g}$$

For the 'normal' risk product,

$$H_o - \Sigma R + \Sigma I = -4.98 - 0.92 + 0.6$$
$$= -5.50 \log \text{cfu/g}$$

For the 'general' product sampled at retail,

$$H_o - \Sigma R + \Sigma I = -5.05 - 0 + 0.5$$
$$= -4.55 \log \text{cfu/g}$$

The 'target' FSO in all cases is −5.31 log cfu/g.

In the former case, the results suggest that less stringent testing could achieve the level of confidence required that the product meets the FSO while in the second case for 'normal' risk product, a further reduction of 0.19 log cfu/g is required to achieve the FSO. In the latter case, for the 'average' product available to U.S. consumers a reduction of 0.76 log cfu/g on average is required.

17.4.2 Exploring Alternative Risk Management Options

Considering the latter case, four options are available to meet the FSO:

(i) decrease H_0
(ii) increase the efficacy of disinfection of the product
(iii) decrease the potential for growth of EHECs on the product during distribution and marketing
(iv) microbiological testing

Table 17.2 Comparison of efficacy of technologies for inactivation of EHECs on leafy vegetables (Niemera, *pers. comm.*, 2015)

Treatment regime	log cfu inactivation
Washing + chlorine	0 to 1.5
Washing + other sanitizers	0 to 4
Cold-plasma and variations	2 to 6
Sonication	0.3 to 1
Washing + sanitizer + sonication	0.5 to 2

17.4.2.1 Decreasing H_0

Practices for leafy vegetable production to minimize contamination on product entering the processing plant are discussed below (Sects. 17.5.2 and 17.5.3). It is not possible to reliably quantify the efficacy of those strategies but a producer could identify improvements that could assist in reducing H_0 and so to increase the probability that the FSO will be achieved. Importantly, as demonstrated earlier, the required H_0 is so low that it cannot feasibly be confirmed by routine, batch-by-batch testing but can only be demonstrated with confidence by accumulation of tens of thousands of negative results.

17.4.2.2 Increasing the Efficacy of Disinfection

From the calculations presented above, Table 17.2 presents a summary of reported efficacy of various disinfection treatments for EHECs on RTE leafy vegetables. From the data presented in the Table 17.2, processors could evaluate the relative efficacy of their current pathogen load reduction methods to other technologies either to replace or augment those technologies to achieve additional pathogen reduction, of approximately 0.3 log cfu/g. For example, a processor using chlorine as their sanitizer might consider changing to a different chemical sanitizer, e.g., perchloroacetic acid, which would expected to achieve higher levels of inactivation of EHECs.

17.4.2.3 Decreasing the Potential for Growth of EHECs on the Product

Most RTE leafy vegetables products are sold without preservatives or packaging that retards residual plant metabolism or microbial growth. As such, temperature control is the only means to manipulate the potential growth of EHECs on the product. From the analysis presented above, temperature would need to be reduced to reduce the expected growth in the distribution chain by ≥ 0.27 log cfu. This might be achieved by either preventing higher temperatures, where the growth rate is faster, or by reducing the average temperature during distribution and storage. To place the magnitude of the temperature reduction required to achieve the desired reduction in growth into perspective, assuming a 10 day shelf life of product after it is received by the retail store, and using the growth rate model developed by Danyluk and Schaffner (2011), i.e.,

$$\begin{aligned} &\text{if Temperature}\left(^{\circ}\text{C}\right) \leq 2.628, \text{then}: \\ &\qquad \text{Growth rate}\left(\log \text{cfu} / \text{day}\right) = 0 \ \text{ and} \\ &\text{if Temperature}\left(^{\circ}\text{C}\right) > 2.628, \\ &\qquad \text{Growth rate}\left(\log \text{cfu} / \text{day}\right) = \left[0.0616^{*}\left(\text{Temperature}\left(^{\circ}\text{C}\right) - 2.628\right)\right]^{2} \end{aligned} \tag{17.3}$$

While *E. coli* do not usually grow at temperatures less than 7 °C, temperatures typically fluctuate in distribution chain potentially allowing periods of growth. The estimated average storage temperature associated with a 0.6 log cfu/g increase in EHEC levels over 10 days is 6.6 °C. In the second example, reducing the predicted growth by 0.19 log cfu/g over 10 days would achieve the FSO. To do so, the average storage temperature would need to be 5.9 °C; a reduction in average storage temperature of ~0.7 °C is estimated to result in achieving the FSO.

In the third case involving the average product, temperature control alone could not achieve the FSO and further actions to decrease the H_0 or to increase inactivation or removal of EHECs from the product after harvest would be required. A further reduction of 0.76 log cfu/g is required and could be achieved

by adoption of a more effective sanitizer, or some combination of washing, sanitizer and/or physical inactivation/removal method such as sonication or cold plasma, as suggested by the data in Table 17.2. Improved practice prior to harvest could also contribute to reduction of H_0.

17.4.2.4 Testing

Testing might also be considered as a means of reducing the overall contamination in leafy vegetables consumed by identifying and removing contaminated batches before they enter the retail supply chain. This possibility will be considered in Sect. 17.4.3.

17.4.2.5 The Significance of Cross-Contamination

The importance of sanitizers in wash water to prevent cross-contamination of product during washing has been (Sect. 17.3.4.1) and will be (Sect. 17.5.1) discussed. Cross-contamination of EHEC between leaves contaminated in the field and uncontaminated leaves may occur during the washing of leaves when no sanitizing chemicals are present. The examples presented below assume that there are no sanitizers in the wash, and that all EHEC entering the system are either in the wash water or redistributed onto previously uncontaminated leaves.

As discussed above, the required H_0 is so low that it cannot feasibly be confirmed by routine, batch-by-batch testing. For example, if we assume that 5 leaves (12.5 g) in a batch of 182 kg of product are contaminated, it is very unlikely that any one of these 5 leaves will be detected during preprocess testing. In fact, the probability can be calculated from Eq. 17.2. If a leaf weighs 2.5 g, and a batch is 182 kg, there are ~72,800 leaves in a batch and the probability of selecting any of those 5 leaves is 5/72,800. If the high risk sampling is used, there are 300 chances to select one of those leaves while if the 'normal' risk testing is used, there are 60 chances to select one of those leaves. Using Eq. 17.2, the probability of detecting the contamination before processing is 2% using the 'high' risk sampling scheme, while for the 'normal' risk product the chance of detecting the contamination is only 0.4%. If we assume each of these 5 leaves is contaminated with 0.2 g feces each, the feces contains 6 log cfu/g EHEC, and the batch is washed in 5000 L of water that contains no sanitizing chemical, 90% (1 log) of the EHEC (900,000 cfu) will be transferred into the wash water (5000 L). These would result in uniformly distributed EHEC in wash water at a concentration of 0.180 cfu/ml. Testing of the wash water, following a concentration step, could be a means of evaluating presence of EHEC in a batch, without testing product post-processing when sanitizer is lacking in the wash water. If the washing system does include a sanitizer, care must be taken to inactivate the sanitizer during the water sampling to avoid an artificially low result due to sanitizer activity on the EHEC between sampling and sample processing. Detection of any EHEC in this situation would indicate potential contamination of the entire batch.

Assuming the remaining 10% of EHEC entering the system (100,000 cfu) from the five initially contaminated leaves are uniformly redistributed over all leaves that pass through the wash system, the EHEC concentration post-washing is 0.549 cfu/g (1.38 cfu/leaf) and should be detectible by 'normal' (60 leaves) or 'high risk' (300 leaves) sampling protocols. While routine testing of product post-processing is typically not recommended, the washing process could be expected to redistribute EHEC from a few highly contaminated leaves more uniformly among all leaves in the system. This would give greater confidence that the test results were representative of the overall contamination in the batch and that highly concentrated but infrequent contamination would be detected. This is considered in greater detail below and including Table 17.3.

Table 17.3 Probability of detecting EHEC following different cross-contamination scenarios

No. leaves	Fecal matter (g/leaf)	EHEC in feces (cfu/g)	EHEC on leaf post processing (cfu/g)	P_{detect} (n = 150)	P_{detect} (n = 60)
5	0.2	1,000,000	0.549	1	1
		10,000	0.00549	1	1
		1000	0.000549	1	0.999
	0.05	1,000,000	0.137	1	1
		10,000	0.00137	1	1
		1000	0.000137	0.999	0.958
1	0.2	1,000,000	0.110	1	1
		10,000	0.0110	1	1
		1000	0.000110	0.998	0.920
	0.05	1,000,000	0.0274	1	1
		10,000	0.000274	0.999	0.998
		1000	0.0000274	0.788	0.462

As the assumptions in the example above (e.g. 6 log cfu/g EHEC in feces, 0.2 g fecal matter on contaminated leaves, 5 contaminated leaves per batch) may represent worst-case scenarios, not expected in the day-to-day operations of RTE leafy vegetable processing, other scenarios were explored. Table 17.3 shows the probability of detecting EHEC by 'normal' (60 leaves) or 'high risk' (300 leaves) sampling protocols, based on different inputs of number of leaves contaminated, amount of fecal matter per leaf, and concentration of EHEC in the incoming fecal matter. Here, the value of post-process testing is also demonstrated, as in all scenarios except one (1 contaminated leaf, 0.05 g feces, and 3 log cfu/g EHEC in feces), there is a greater than 92% probability to detect EHEC. However, when one considers the PO of −5.91 log cfu/g, the probability of detecting EHEC by either 'normal' (60 leaves) or 'high risk' (300 leaves) sampling protocols are 6.84% and 16.2%, respectively. These low detection probabilities highlight the importance of contamination prevention in the field, exclusion of contaminated crops entering the processing stream (Sects. 17.1 and 17.5.3) and preventing cross-contamination in washing systems (Sects. 17.3.4.1 and 17.5.1).

17.4.3 Stochastic Approaches

Deterministic calculations (e.g., those based on averages or some other single representative measure of each variable, e.g. 95th percentile) can lead to poor risk management decisions because the single values or risk estimates that are derived are often not representative of the range of possible outcomes. This was discussed and exemplified by Nauta (2000). Relevant to the current example, both Danyluk and Schaffner (2011) and Pérez Rodríguez (2011, *pers. comm.*) based their analyses on data from Ecosure (2008) which gives mean temperatures and ranges of temperatures for:

(i) retail back room refrigerators,
(ii) retail display refrigerators, and
(iii) home refrigerators.

Using the distribution of temperatures and times of storage in those stages of the retail-to-consumption sections of the supply chain, both studies estimated growth in that section of the farm-to-fork chain as ~0.6 log cfu/g. However, the average temperatures in refrigerators in each of those sections was 2.2 °C, 4.4 °C and 3.4 °C, respectively. As noted earlier, *E. coli* does not usually grow at temperatures < 7 °C. Accordingly, had average temperatures been used in the calculations, no growth would have been

predicted in those studies. Instead, both studies used distributions of actual temperatures meaning that some growth was predicted in some cases because temperatures exceeded the lower limit for EHEC growth. The magnitude of the temperature variations was 2.5 to 4 °C (SD), meaning that, on ~20% or more of occasions, storage temperatures might allow growth of EHEC on the product despite that, based on the observed average temperatures, no growth of EHEC would be predicted.

Recognizing the consequences of variability in conditions on microbial behavior in foods ICSMF developed a simple spreadsheet tool to help to explore the consequences of variability on food safety risks. The tool can be downloaded from http://www.icmsf.org/main/software_downloads.html. Examples are presented now to demonstrate the significance of including variability in calculations leading to risk management decisions.

Figure 17.3 shows a normal distribution of cell concentrations with the desired performance objective set as the mean of that distribution. The spread of the distribution is given by a standard deviation of 1.4 log cfu/g. The standard deviation is representative of the very inhomogeneous distribution of contaminants that might be expected on leafy vegetables, as was considered in Sect. 17.4.2.5. From that distribution, we can estimate the proportion of samples of different size that would be expected to contain one or more EHEC cells, and that could be detected by an enrichment technique. For this example we consider sample size of 25 g and 100 g. For a normal distribution with mean −5.9 and standard deviation 1.4, about 0.267% of samples are expected to contain a level above −2 log cfu/g. In our example, this means that approximately one in 375 samples of 100 g would be expected to contain a cell (i.e., have a concentration in excess of −2 log cfu/g). Similarly, one in approximately in 1538 samples of 25 g would be expected to contain a cell (i.e., have a concentration in excess of −1.67 log cfu/g).

From Eq. 7.3 we can then calculate how many 25 g or of 100 g samples would need to be tested, and be shown not to contain an EHEC, to provide 95% confidence that the average concentration in the batch was ≤ −5.9 log cfu/g. The number of 25 g samples required is 4604 (or ~115 kg of product) and for 100 g samples, 1121 samples (~110 kg of product) need to be tested and found not to contain EHEC. Using 750 g samples, 194 samples would need to be tested to give the same level of assurance.

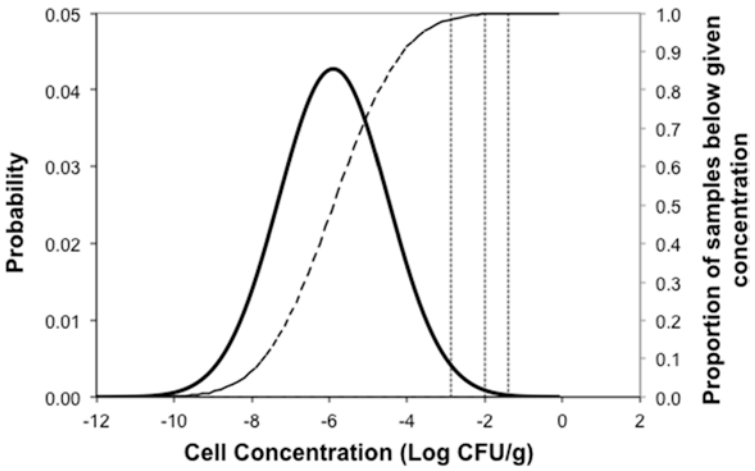

Fig. 17.3 An example of a distribution (bold solid line) of expected cell counts in a batch of RTE leafy vegetables. The distribution shown has an average concentration of −5.9 log cfu/g and is characterized by a standard deviation of 1.4 log cfu/g, considered to be representative of an inhomogeneous product. Also shown is the cumulative distribution (dashed line) indicating the proportion of samples expected to have concentration below that shown. The three dotted lines indicate different sample sizes (750 g, 100 g and 25 g) to highlight the proportion of samples that would be expected to have one cell or more in a sample of that size

As an additional example of the benefits of a stochastic approach, we consider the example above of a small number of highly contaminated leaves in a batch that is otherwise clean. For the sake of the example, imagine a batch in which 5 leaves are contaminated with 0.2 g each of feces containing 1000 cfu/g. The contamination in the remainder of the batch (72,800 leaves) is, on average, assumed to be 10^{-5} log cfu/g. In total the batch contains:

$$181{,}987.5\,\text{g} \times 10^{-5}\,\text{cfu/g} + 12.5 \times 10^{3}\,\text{cfu/g} = 1002\,\text{cells among}\,182{,}000\,\text{g}.\text{Thus,}$$
$$\text{the average} = -2.26\,\log\,\text{cfu/g}.$$

While the distribution of cells among the vast majority of the leaves is low, there are 5 leaves that are highly contaminated, and as discussed above, highly unlikely to be sampled. Thus, we assume a standard deviation of 1.4, as in the above example. However, if washed without an appropriate intervention for preventing cross-contamination, the standard deviation of the distribution would be reduced (i.e., contamination would become more homogenous), and the average concentration also reduced, as depicted in the Figure 17.4a. From 17.4b it can be seen that even though washing decreases

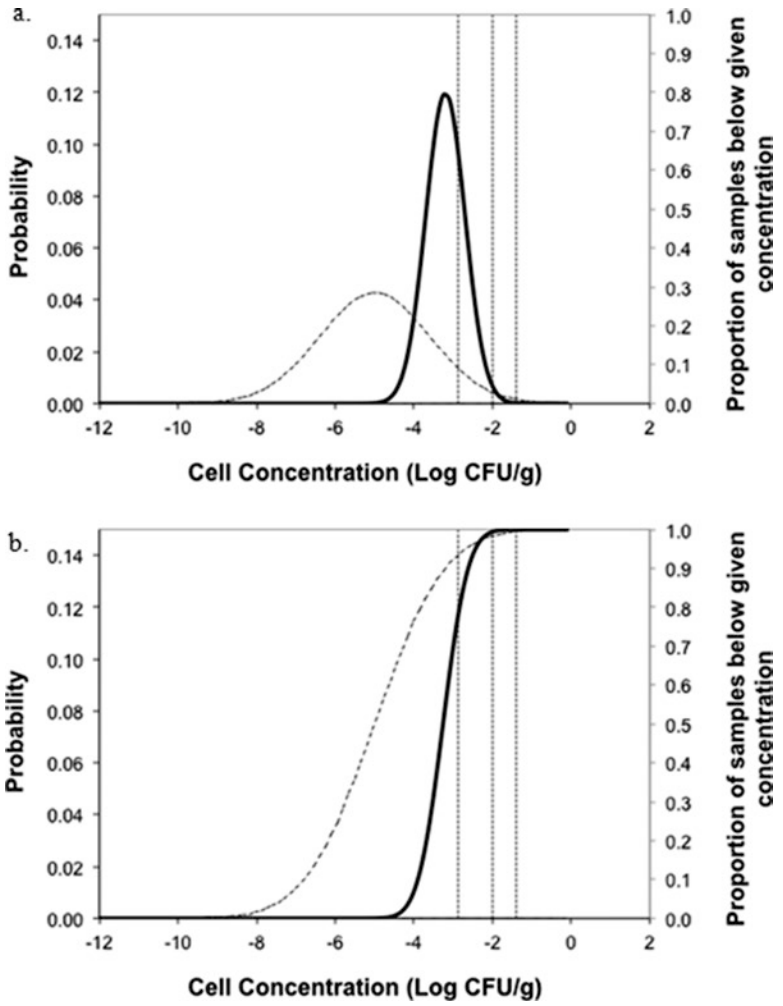

Fig. 17.4 (**a**) Distribution of cells on leaves containing a small number of highly contaminated leaves prior to washing and on the leaves after washing. (**b**) Cumulative distributions for the distributions in (**a**); despite the lower mean concentration and narrower distribution, contamination after washing is readily detectable

the average contamination level on the product, and that the distribution becomes more homogenous throughout the batch, the redistribution of contamination means that the probability of detecting the contamination in a sample increases.

17.5 Relating Risk Management Metrics to Stringency of Food Safety Systems

17.5.1 HACCP Plans

The use of HACCP principals is recommended for fresh-cut leafy vegetables to proactively minimize microbial food safety hazards. Specifically, building safety into processing operations, such as defining sanitizer concentrations in operations involving reticulated water, to control opportunities for cross-contamination of EHEC through a facility, could be considered a CCP and managed as such. A generic model HACCP plan for RTE fresh-cut vegetables has been developed and is available from the Canadian Food Inspection Agency (CFIA 2015).

17.5.2 GHP Programs

Certain aspects of GHPs must be controlled to minimize contamination or recontamination of fresh-cut products with pathogens other than EHEC. Codex guidelines (CAC 2003b) on the Code of Hygienic Practice for Fresh Fruits and Vegetables provides a framework for control of foodborne pathogens for fresh produce.

17.5.3 GAPs

Potential sources, routes of contamination and other risk factors leading to contamination of produce in the agricultural environment include irrigation and other waters (Benti et al. 2014; Harris et al. 2012; Holvet et al. 2014; Wood et al. 2010), compost (Franz et al. 2008), soil (Ponce et al. 2008), feral animals (Jay et al. 2007), domestic animals (Hutchison et al. 2008), and farm management/harvest practices (Strawn et al. 2013). Outbreak investigations, field trials, and laboratory-based research have clearly established the importance of GAPs for preventing initial contamination and subsequent amplification of pathogenic microorganisms.

17.5.4 Regulatory Requirements and Criteria

Presently, there are no internationally harmonized approaches to evaluating the microbiological safety of fresh-cut leafy vegetables. In the U.S., the 2011 Food Safety Modernization Act (FSMA) legislation enhances the U.S. Food and Drug Administration's (FDA) ability to require certain specific safety standards for growers, harvesters, and facilities that manufacture, process, pack, or hold food products including fresh-cut leafy vegetables. The requirements of FSMAs Produce Safety Rule are built upon the FDA's previous guidance for GAPs and Good Handling Practices for fresh fruits, vegetables, and nuts, and establish specific metrics related to the microbiological quality of agricultural water that contacts the harvested surface of the fruit, biological soil amendments, and raw manure use. Fresh-cut

leafy vegetable operations also fall under FSMAs Preventive Controls (PC) for Human Foods Rule (U.S. FDA 2015). Under the PC rule, all facilities are required to develop a Food Safety plan that includes a Hazard Analysis to identify and address hazards that require a preventive control, and includes a recall plan if hazards are identified.

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Chapter 18
Viruses in Oysters

18.1 Introduction

Seafood constitutes an integral part of the human diet particularly in coastal regions and island nations. According to FAO, in 2013 the average annual per capita fish and shellfish consumption in the world was 18.98 kg (FAO 2017). Shellfish production (molluscs and others) has grown substantially in the last 50 years, from 1.9 million metric tons in 1961 to 17.5 million in 2013. The supply of molluscs in U.S. in 2013 was 1,057,882.44 ton with per capita consumption of 3.31 kg/year (FAO 2017).

Bivalves, as a food, are characteristically tender, easily digested, additive-free, have high-quality animal protein and are minimally processed. These characteristics make them a desirable product and an important component in the human diet worldwide. Although shellfish can serve as a source of high quality animal proteins, they can also harbor some of the most pathogenic microorganisms and potentially lethal toxins. Bivalve shellfish are often minimally processed, and can be a high public health risk. The commonly encountered pathogenic microorganisms in bivalves include bacteria like *Vibrio parahaemolyticus*, *V. cholerae*, *Salmonella enterica*, *Clostridium* spp., *Campylobacter* and viruses such as hepatitis A, norovirus, poliovirus, astrovirus, rotavirus, coxsackie B, and echovirus (Koopmans and Duizer 2004). Single-cell algae, mostly dinoflagellates, which produce a range of toxins that induce neurological symptoms or gastrointestinal disorders in humans can also be concentrated by shellfish with the toxins remaining active in the oyster for weeks to months.

Human disease transmission due to the consumption of shellfish contaminated by viruses was first recognized in the 1950s with the report of a large outbreak of clam-associated hepatitis A (Roos 1956). The safety of shellfish is generally affected by the natural aquatic environment, the anthropogenic changes in the natural environment and the catching, handling, processing, storage and transportation process.

Bivalve shellfish are generally sedentary animals that obtain subsistence by filtering nutrients from the water at a rate of up to 10 L/h. These filter feeders not only ingest the suspended algae that serve as their primary food but also bacteria and viruses that may be present in the aquatic milieu. Bivalves have been shown to bioaccumulate and concentrate viruses up to 100-fold compared to their surroundings (Nappier et al. 2008). Shellfish are mostly cultivated in coastal and estuarine waters that may be exposed to contamination by sewage. The majority of foodborne outbreaks related to shellfish are associated with the consumption of shellfish from contaminated waters that are polluted by human excreta or affected by toxic microalgal blooms. Infected or asymptomatic food handlers can also cause contamination of the food.

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The magnitude of the problem increases because many shellfish are generally eaten raw or only partially cooked, and the two commonly used methods to make the bivalves safe for consumption, i.e. relaying and depuration, are not effective in purging out the viruses as compared to fecal coliforms and pathogenic, enteric bacteria (de Medici et al. 2001; Nappier et al. 2008).

18.2 Risk Evaluation

18.2.1 Hazard Identification/Risk Profiles

The scope of this chapter is limited to shellfish-associated human viruses, particularly norovirus and hepatitis A virus.

18.2.1.1 Norovirus

Noroviruses (previously referred to as Norwalk-like viruses [NLVs] or small round-structured viruses [SRSVs]) are small round viruses belonging to the genus Norovirus of the Caliciviridae family. The size of the virion is 27–38 nm in diameter, with a single-stranded, positive-sense, polyadenylated RNA genome. The genus Norovirus is highly diverse with approximately 46% nucleotide divergence across the genome. They are divided into five genogroups (GI–GV) based on amino acid identity in the major structural protein (VP1) (Zheng et al. 2006). Each genogroup has been further divided, on the basis of > 85% sequence similarity in the complete VP1 genome, into at least eight genotypes belonging to GI and 21 genotypes belonging to GII (Wang et al. 2005; MMWR 2011). The strains belonging to different genogroups have some host specificity with human infection reported from GI, GII and GIV strains, bovine and ovine infections with GIII strains (Oliver et al. 2003; Wolf et al. 2009), porcine infections with GII strains (Green et al. 2000; Wang et al. 2005), dogs and lions by GIV (Fankhauser et al. 2002; Martella et al. 2007, 2008) and mice by GV (Karst et al. 2003). A sixth genogroup has also been recently proposed, with infections reported in both humans and dogs (Mesquita et al. 2010). The animal noroviruses have not yet been isolated from humans, but human noroviruses have been isolated on several occasions from animals (Mattison et al. 2007; Martella et al. 2007; Scipioni et al. 2008), which suggests the potential for zoonotic transmission. It has yet to be determined if human NoVs frequently infect animals that act as a reservoir, or whether these transmissions are rare zoonotic events. Shellfish may act as a mixing vessel for the emergence of recombinant NoV strains following contamination with multiple strains (Bosch et al. 2008).

18.2.1.2 Hepatitis A Virus

Hepatitis A (HAV) virus belongs to genus Hepatovirus of the Picornaviridae family. It is an icosahedral virus, which has a single-stranded, positive-sense RNA genome of approximately 7.5 kb. Immunological evidence has determined the existence of a single serotype of HAV, although several genotypes can be differentiated by molecular methods based on the putative VP1 / 2A junction. Initially, seven genotypes were identified: genotypes I, II, III and VII associated with human infections and genotypes IV, V and VI in simians. However, recent publications have re-classified genotype VII as a sub-genotype of genotype II (Costa-Mattioli et al. 2002; Lu et al. 2004). Genotypes I and III are the most prevalent genotypes isolated from humans. Subtype IA is responsible for the majority of hepatitis A cases worldwide, whereas subtype IB has been mainly found in the Mediterranean region (Nainan et al. 2006; Pinto et al. 2007), with reports from other areas as well (Sánchez et al. 2002).

18.2.1.3 Epidemiology

Estimating the burden of foodborne viral diseases is difficult because the majority of viral gastroenteritis cases are mild and go unreported. In addition, both norovirus and hepatitis A virus have a tendency for person-to-person spread thereby making it difficult to pin-point the exact extent of the cases that are directly foodborne. Data collected from diverse sources estimated the proportion of foodborne viral illnesses to be nearly 5% for HAV and 12–47% for NoV, which translates to approximately 13,000 to 30,000 per million persons (FAO/WHO 2008c). A study of the burden of foodborne disease in New Zealand ranked NoV among the top three etiologies of gastroenteritis (Cressy and Lake 2007). Data from Australia, The Netherlands, United Kingdom and New Zealand attribute 31.6% (Hall et al. 2005), 10–20% (de Wit et al. 2003; Kreijl et al. 2006), 8% (Adak et al. 2002) and 14.4% (Lake et al. 2000) of the foodborne gastroenteritis cases to viral agents. Limited information is available from Asian countries. The Ministry of Health, Welfare and Labor in Japan places viral agents as the leader in foodborne illnesses with 48.2% of the reported cases (FAO/WHO 2008). Crustaceans, molluscan shellfish and their products were the most frequently implicated food items in foodborne viral illnesses caused by NoV and HAV (EFSA 2011), a situation reflected in U.S, with at least 40 NoV outbreaks linked to oysters from 2005 to 2017 (CDC 2017a).

In Europe, norovirus has been linked to several oyster related outbreaks (Doyle et al. 2004; Le Guyader et al. 2006a; Nenonen et al. 2009). During 2000–2007 nearly 17.5% of the internationally reported norovirus outbreaks were due to bivalve shellfish (Baert et al. 2009). Between March 2006–2010 the European Rapid Alert System for Food and Feed (RASFF) database revealed 19 alert notifications on norovirus findings in oysters or food poisoning associated with the consumption of oysters.

Hepatitis A infections have a worldwide distribution, occurring in both epidemic and sporadic fashion. In the U.S., from 1980 to 2001, an average of 25,000 cases of hepatitis A was reported to the Centers for Disease Control and Prevention (CDC) annually. However, correcting for under-reporting and asymptomatic infections, CDC estimated that an average of 263,000 HAV infections, from all causes, occurred annually in the U.S. during this period (FDA 2012b).

Hepatitis A is a significant public health problem in developing nations with approximately 1.4 million new infections per year (WHO 2000). Hepatitis A is the most serious viral infection associated with shellfish consumption. The first documented shellfish associated infectious hepatitis outbreak was reported in Sweden in 1955 with 629 cases (Roos 1956). The largest viral food poisoning outbreak attributed to hepatitis A contaminated shellfish occurred in 1988 in Shanghai, China, affecting nearly 300,000 people (Halliday et al. 1991). There are several documented outbreaks associated with shellfish consumption throughout the world (Conaty et al. 2000; Bialek et al. 2007; Pontrelli et al. 2007; Shieh et al. 2007). Shellfish have been identified in Italy to be the most important source of foodborne hepatitis A infections, with 70% of all hepatitis A cases linked to shellfish consumption (Mele et al. 1990, 1991; Salamina and D'Argenio 1998). In the U.S., hepatitis A outbreaks linked to shellfish had been reported frequently, but the incidence has decreased significantly with no major outbreak being reported between 1989–2004 (Desenclos et al. 1991; Glass et al. 1996; Bialek et al. 2006; Shieh et al. 2007), and only one since 2005 in U.S. attributed to oysters (CDC 2017a) and one suspected outbreak in Hawaii, linked to raw scallops, in 2015 (FDA 2017).

18.2.2 Factors Affecting Hazard Characterization

18.2.2.1 Norovirus Infection

Norovirus infection is usually a self-limited illness; healthy persons typically recover without sequelae. Typical symptoms include sudden onset of vomiting and watery diarrhea. Other clinical manifestations are nausea, abdominal cramping and pain, malaise, anorexia, fever, chills, headache

and myalgia. Complications can occur in the elderly, chronically ill and immunocompromised patients, particularly recipients of solid organ and stem cell transplants, and include volume depletion, electrolyte disturbances (e.g., hypokalemia), and renal insufficiency (Nilsson et al. 2003; Mattner et al. 2006; Roddie et al. 2009; Westhoff et al. 2009). Some unusual complications have been described in special populations, such as disseminated intravascular coagulation in previously healthy soldiers exposed to severe environmental stresses (Atmar 2010), necrotizing enterocolitis in a neonatal intensive care unit (Turcios-Ruiz et al. 2008), benign infantile seizures among Chinese children hospitalized with acute gastroenteritis (Chen et al. 2009), exacerbation of inflammatory bowel disease (Khan et al. 2009) and death among elderly residents of nursing home facilities (CDC 2007).

Genetic factors and acquired immunity play a role in susceptibility to NoV infection. Acquired immunity following a symptomatic infection is not long lasting (2–3 years), and symptomatic illness develops following a subsequent re-challenge. Immunity is also strain specific; exposure to a serologically distinct strain leads to symptomatic infections (Wyatt et al. 1974; Atmar 2010). Studies on the potential role of host susceptibility indicate that norovirus binds differentially to histo-blood group antigens (HBGAs), and that the binding pattern correlates with susceptibility to infection and illness (Hutson et al. 2002, 2005; Lindesmith et al. 2003). People expressing a blood group B antigen are less likely to become ill following challenge with Norwalk virus (Hutson et al. 2002, 2003). Individuals who are secretor-negative (SE-) for fucosyl transferase-2 (FUT-2), an enzyme important in the synthesis of HBGAs, are resistant to infection with GI.1, GII.3 and most GII.4 NoVs. Individuals with blood group O or A are secretor-positive (SE+) and have increased susceptibility. However, SE- individuals can be infected with Snow Mountain virus, a GII.2 strain (Lindesmith et al. 2005), and GI.3 strain (Nordgren et al. 2010). The binding pattern of NoVs to HBGAs varies between genotypes (Huang et al. 2005; Shirato et al. 2008), and even within a genotype (Lindesmith et al. 2008). Thus, it is likely that every person is genetically susceptible to one or more NoV genotypes (Atmar 2010).

Certain genotypes are more likely to be associated with specific routes of transmission. For example, GII.4 strains are more commonly associated with person-to-person transmission, while GI strains are identified more frequently in shellfish-associated outbreaks (Siebenga et al. 2007; Le Guyader et al. 2006a). The virus is shed in high numbers in feces, e.g., 10^8 to 10^{11} RNA copies/g of stool (Atmar et al. 2008). Projectile vomiting which is commonly associated with norovirus infection can disseminate about 30 million virus particles in the environment during a single episode (Barker et al. 2004; Aoki et al. 2010). This is especially relevant in the case of food handlers and shellfish harvesters.

Development of dose-response curves for NoV is complicated by differences in susceptibility in SE+ and SE- individuals, differences among NoV strains, and apparent differentials between levels needed to produce an infection versus levels to produce illness. For example, analysis of data from oyster related outbreaks indicated that for SE+ individuals the probability of infection from a single genome copy of NoV GI and GII was 0.29 and 0.40, respectively, while the probability for illness was 0.13 and 0.18, respectively (Thebault et al. 2013). On the other hand, SE- individuals were strongly protected. Teunis et al. (2008) estimated the ID_{50} for illness was approximately 6000 genome copies with the probability of illness increasing with the dose (de Wit et al. 2007; Visser et al. 2010). Determination of accurate dose-response curves for NoV is complicated by their propensity to aggregate, which could account for an underestimation of the probability of infection and illness by an order of magnitude (Teunis et al. 2008; McBride 2014). EFSA (2012) reported that exposure of human volunteers to serial dilutions yielded a dose-dependent probability of becoming ill ranging from 0.1 (at a dose of 10^3 NoV genome copies) to 0.7 (at a dose of 10^8 virus genome copies). Similarly, Teunis et al. (2008) estimated that the dose required to cause illness was between approximately 1000 infectious units (for aggregated viruses) or 1 million particles for disaggregated NoV administered in human challenge trials.

In a study in U.K., a statistically significant difference was found in norovirus RNA levels in oyster samples strongly linked to norovirus or norovirus-type illness (1048 copies/g) with the levels

(121 copies/g) typically found in commercial production areas (non-outbreak-related samples). Further, none of the outbreak-related samples contained fewer than 152 copies/g, the majority of positive results for non-outbreak-related samples was below this level (Lowther et al. 2012a). However, human cases have been reported after the consumption of less than 100 RNA copies/g of the shellfish digestive tissue (Baker et al. 2011; Lowther et al. 2010). Considering the high infectivity estimated at levels as low as single genome copy per serving, it can be anticipated that a lower dose extrapolation (e.g., 1 genome copy/10 serving, 1 genome copy/100 servings) would be linear.

18.2.2.2 Hepatitis A

The incubation period for hepatitis A is between 2 and 6 weeks, with an average being 28–30 days. Virions are shed during the incubation period and for up to 2 weeks following the onset of symptoms. The symptoms of hepatitis A infection include fever, loss of appetite, nausea, vomiting, and abdominal discomfort, followed by jaundice. Symptoms usually last less than 2 months, although some people can be ill for as long as 6 months (FDA 2012b). In infants and children younger than 5 years of age, infection is often mild or asymptomatic. Older people and those with chronic liver disease are more likely to have severe manifestations of hepatitis A. The case-fatality rate among cases reported through U.S. national surveillance reaches a high of 1.8% among people ≥ 60 years, and fulminant hepatitis has been reported more frequently among older patients with hepatitis A (CDC 2017b). Immunity to hepatitis A is usually lifelong (Hollinger and Emerson 2007). Some mutations in the HAV genome at the 5′NCR or at the VP1X2A and 2C regions are reported to be associated with fulminant hepatitis (Fujiwara et al. 2001, 2002, 2003) and higher virulence in tamarinds (Emerson et al. 2002), respectively.

The infectious dose is estimated to be 10–100 virus particles for hepatitis A (FDA 2012b). The virus, like norovirus, is shed in high concentrations in the feces even before the onset of symptoms in infected individuals. HAV is known to persist on fomites for extended periods (Abad et al. 1994). These two factors along with the extended shedding period make secondary spread an important aspect of the virus' infectivity.

18.2.3 Factors Affecting Exposure Assessment

18.2.3.1 Sources and Routes of Oysters Contamination

Major sources of contamination of oysters at the site of production or harvest include untreated effluent, storm/rain-water runoff, combined sewer over-flow (CSO), livestock slaughterhouse and processing effluent, over-flow from manure lagoons and seasonal influxes due to seasonal population highs when wastewater treatment systems may be overwhelmed. Factors affecting the presence and survival of viruses in shellfish include exposure time, virus concentration in the water, presence of particulate matter, temperature, pH, salinity, food availability, type of virus and individual shellfish variation (Sobsey and Jaykus 1991). Post-harvest contamination can occur by human handlers through direct contact during handling or indirect contact, such as from clothing, movement, generation of aerosols or sneezing. These events may occur during collection, processing, distribution or preparation of shellfish and shellfish products. Viruses attached to particles, like dust or aerosolized droplets, may land on food contact surfaces or directly onto the food product. In such cases the number of viruses on each particle is generally low, and their distribution is affected by factors like air movement, relative humidity, degree of product exposure, concentration of particulates in the air. Post-harvest contamination is generally considered to be of minor importance in the overall likelihood of contamination of oysters with viruses.

An important factor that influences exposure assessments of foodborne cases is the ability of the NoV and HAV to spread from person to person via contact. The viruses are shed in the feces of infected individuals at levels $\geq 10^8$ virions/g of feces. In a volunteer study on norovirus infection, peak virus titer levels occurred at 2–5 days after inoculation, with virus shedding first detected using reverse transcriptase-PCR (RT-PCR) 18 h after inoculation and lasting an average of 28 days (range 13–56 days) (Atmar et al. 2008). Viral shedding in asymptomatic patients is another factor associated with secondary spread of the virus. In a study in The Netherlands, NoV was detected in 5.2% of the healthy controls in the general population (de Wit et al. 2001) and 19% of healthy controls in outbreak settings (Vinjé et al. 1997). Virus shedding in HAV starts 10–14 days before the start of symptoms thus potentially exposing a large number of susceptible individuals to the hazard.

18.2.3.2 Levels of Virus in Oysters and Prevalence

The prevalence of NoV and HAV in oysters and other molluscan shellfish has been studied extensively in different parts of the world. The prevalence rates vary from place to place and season to season. However, reports on the levels of viruses in oysters are limited (EFSA 2012). Boxman (2010) compiled data on the occurrence of human enteric viruses in shellfish from European markets; 0–90% of shellfish samples harbored enteric viruses. A 2-year systematic survey conducted in U.K. from 2009–2011 reported 76.2% of oyster samples from 39 production areas were contaminated with norovirus (Lowther et al. 2012b). In a similar survey conducted in 9 states of U.S. in 2007, NoV or HAV were detected in < 5% of retail oysters (DePaola et al. 2010).

18.2.3.3 Seasonal Influence

The majority of shellfish associated gastroenteritis cases occur during the winter months and only rarely during the summer months. The majority of virus detections in oysters were between October and March in Europe (Lowther et al. 2008; EFSA 2012). Nearly 78% of the shellfish-associated illness in U.S. during 1991–1998 occurred between November and January (Burkhardt and Calci 2000). This may partly be due to climatic events such as more rainfall during January to April, with increasing risk of sewage input into coastal environments (Maalouf et al. 2010a). Another likely explanation is the decreased physiological activity of shellfish below certain temperatures. The pumping activity of shellfish is greatly reduced below 2 °C, and rises, as the temperature increases from 8 °C to 28 °C, with pumping rates and feeding decreasing when the temperature exceeds approximately 35 °C (Shumway 1996). The reduced exchange of water can lead to reduced elimination of the viruses from the filter feeders.

18.2.3.4 Consumer Practices and Consumption

The most important factor responsible for shellfish-related outbreaks is the consumption of raw or undercooked shellfish. The enteric viruses are highly resistant to acidic pH and heating. HAV was found to retain its infectivity after exposure to pH 1 for 5 h and 90 min at room temperature and 38 °C, respectively (Scholz et al. 1989). Hepatitis A virus is inactivated at 85–90 °C for 1 min, with boiling in water for 3 min being better than steaming for 3 min (Millard et al. 1987; Hewitt and Greening 2006). Heating at 85–90 °C for 90 sec is considered satisfactory for the inactivation of most viruses, but may render the shellfish unacceptable for consumption. The usual practice is to heat the oysters until the shell opens, which is insufficient for virus inactivation.

18.2.3.5 Characteristics, Behavior and Survival of Viruses

Enteric viruses are non-enveloped, thus they are highly resistant to the effects of disinfectants, extremes of pH (acidic and alkaline), drying, radiation *etc*. Data on norovirus infectivity, persistence and inactivation are not available because of the inability to culture them using available cell lines. Therefore, model surrogate viruses have been used. These include feline calicivirus (FCV) and the murine norovirus (MuNoV). The former is a respiratory pathogen and hence may not be a good a surrogate for human norovirus. The MuNoV can better tolerate acidic pHs than FCV, a property similar to the human NoV (Cannon et al. 2006) and is thus often used as a surrogate for human norovirus. Norovirus can tolerate pH 2.7 for 3 h at room temperature (Dolin et al. 1972) and can survive heat better than vegetative pathogenic bacteria. The virus is also resistant to a free chlorine concentrations of 0.5–1 mg/L for 30 min, but is inactivated at concentrations greater than 2 mg free chlorine/L. The HAV is typically much more resistant to the commonly used food preservation methods and disinfectants than enteric adenovirus and poliovirus. It has been shown to be the more resistant than other enteric viruses to heat, desiccation, extremes of pH and ionizing radiations (EFSA 2011).

Studies using artificially contaminated finger pads have shown transfer of 10% to 50% of HAV to different foods and other surfaces (Bidawid et al. 2000, 2004). The survival of enteroviruses is favored by high relative humidity while the reverse is seen with HAV and rotavirus (Mbithi et al. 1991). The HAV retained its infectivity for 30 days in dried feces at 25 °C and 42% relative humidity (Hollinger and Ticehurst 1996). Mbithi et al. (1992) reported that 16–30% of HAV could be recovered following 4 h of drying on the finger pads at room temperature. The occurrence of consecutive outbreaks of a similar norovirus strain in cruise ships even after thorough disinfection is an example of the long-term survival of the virus on inanimate objects under adverse conditions (Wu et al. 2005). NoV could be recovered for 4 and 8 weeks at 20 °C and 4 °C, respectively, from both polyvinyl chloride (PVC) and stainless steel (Lamhoujeb et al. 2009). Hepatitis A virus can survive for up to 1 month in dried fecal matter (McCaustland et al. 1982). Treatment of mixed human and animal waste for 7 days prior to disposal only reduced viral titers by 1 $\log_{10}$ at 37 °C (Deng and Cliver 1995). Once introduced into the water environment, the virus can remain infectious for months, associating itself with marine sediment (Arnal et al. 1998; Bosch 1998).

In marine water, the enteric viruses generally associate with clay and other sediments. The binding of virus to marine sediments is increased at acidic pHs. The presence of pollutants in the water can help in the persistence of enteric viruses in the marine environment (La Belle and Gerba 1980; Rao et al. 1984). The inactivation of viruses occurs as a result of thermal inactivation, pH changes, sunlight and other microorganisms present in the water (Ward et al. 1986; Bosch 1995). HAV has been shown to survive in seawater for several weeks without loss of infectivity, with the period being prolonged in colder water temperatures (Bosch 1995; Callahan et al. 1995). The infectivity of HAV in river water, ground water and tap water was retained for 48 days (Springthorpe et al. 1993), 12 weeks (Sobsey et al. 1989) and 60 days (Enriquez et al. 1995), respectively. In a study on the persistence of human NoV in ground water, the virus was detected by real time reverse transcriptase PCR (RT-qPCR) after storage for 1266 days in groundwater, with no significant reduction after 622 days and only a 1.79 $\log_{10}$ reduction by day-1266 (Seitz et al. 2011). Enteric viruses are rapidly taken up by shellfish with virus being detected as early as 1 h after exposure, with peak uptake by 6 h after storage in contaminated water (Abad et al. 1997). As noted earlier, the viral load of the shellfish can be considerably higher than the surrounding water.

18.2.3.6 Effect of Detection Methodologies on Exposure Assessment

RT-PCR and RT-qPCR are routinely used for the detection and quantitation of the viral load in the shellfish samples. The reliability of PCR assays for enteric viruses is limited by factors such as low concentration of viruses in the shellfish tissue, efficiency of the extraction procedure (which varies from 20–100%)

(Hewitt and Greening 2009), presence of PCR inhibitors, and sequence variation in viruses, especially NoV. Furthermore, a positive PCR reaction does not necessarily indicate that infectious viral particles are present, but instead only indicates that the target gene sequences were present and intact.

Shellfish have been found to harbor viruses in numbers below the detection limits of these methods, but high enough to cause disease in susceptible human populations (Bosch et al. 1994; Sánchez et al. 2002; Le Guyader et al. 2003). Extraction and detection methods need to be sensitive enough to detect low numbers of viral particles. The viruses have been reported to localize in the digestive diverticulum of the shellfish (Romalde et al. 1994; Abad et al. 1997) and have been have reported to bind to the N-acetylgalactosamine receptors present in the digestive tract of oysters (Le Guyader et al. 2006b; Maalouf et al. 2010b). The use of the digestive tract instead of the whole molluscs can increase method sensitivity. Processing is also easier, as this tissue is only one tenth of the total shellfish weight. Considering all the above aspects and the lack of suitable cell culture systems for the detection of norovirus, the use of RT-PCR, RT-qPCR or the combination of these PCR techniques with viral capture systems, such as the use of antibodies or cell receptors to separate virions from the environmental matrix, currently provide the most feasible approach for the analysis of the viral genome and quantification of viruses levels.

A major factor limiting the use of literature data to conduct exposure assessments is the absence of globally standardized and validated methods for the detection of viruses in foods. The European Committee of Standardisation (CEN) has developed a standard RT-qPCR method for quantification of HAV and NoV genogroup I (GI) and II (GII) RNA from foodstuffs or food surfaces (ISO 2013). However, this method does not allow quantification of viral particles below 100 genome copies/g of digestive diverticulum. Legislative standards below this level are unlikely to be analytically feasible using present methodologies (EFSA 2012). A method for detecting and enumerating hepatitis A in shellfish has also been described in the Bacteriological Analysis Manual of the U.S. FDA (FDA 2012a).

18.2.4 Risk Characterization

Risk characterization is the integration of the information and data acquired from the hazard characterization and exposure assessment to obtain a risk estimate by providing either a qualitative or quantitative estimate of the likelihood and severity of adverse effects. Additionally, the risk characterization provides description of the uncertainties associated with a risk estimate. A simple example of how risk might be characterized using a deterministic risk assessment of norovirus in molluscan shellfish, based on U.K. data, is provided below.

ID_{50}: Based on data from human volunteer study fitted to an exponential dose-response model (Fig. 4b in Teunis et al. 2008)	1 million particles
Prevalence of norovirus in shellfish (U.K.) (Lowther et al. 2012b)	76.2%
Representative contamination levels per gram (Fig. 1 in Lowther et al. 2012b)	300 virions
Proportion of population consuming oysters (EFSA 2011)[a]	3.40%
Consumption of bivalve molluscs per capita per year (EFSA 2011)	183 g
Consumption of bivalve molluscs per UK consumer per year (EFSA 2011)	5400 g
U.K. population	6.5×10^7
Total consumption (3.4%*65000000*5400 g)	1.2×10^{10} g (12,000 t) (flesh only)
Expected (potential) contaminated servings (assuming a serve is 360 g)	2.53×10^7
Proportion thoroughly cooked (assumes 90% inactivation)	20% (assumed)
Proportion mildly cooked (assumes 10% inactivation)	5% (assumed)
Proportion consumed raw	75% (assumed)

[a]It is unclear from EFSA (2011) whether the consumption indicated is *per capita*, or *per consumer*, in UK. FAO (2017), however, provides estimates of molluscan shellfish consumption in all nations. From this source, using the 5-year average consumption *per capita* for many nations from 2009–2013, it was concluded that the UK *per capita* consumption is 183 g per day, and that consumption among *consumers* of molluscs is relatively much higher, *i.e.,* given that consumers represent only 3.4% of the total population

No. of cases

	Scenario without depuration or high pressure	Scenario with high pressure treatment (assuming 3 log reduction in NoV; *see* Sect. 18.3.2.3)
Expected cases in 1 year in UK from oysters	1.95 million	2000

The estimated annual incidence of Norovirus infection in UK is 3.7 million (Harris et al. 2017)

The above simple calculations are based on many assumptions and lack detailed, relevant, data. In particular, data on infectious viral particles is not available, and most reports relates to the presence of viral genome copies, whether in an infectious unit, or not. However, the effect of a process to reduce contamination levels or the estimated risk is evident.

18.3 Risk Management

The magnitude of shellfish-associated illnesses makes it imperative that strict control measures be exercised at the pre- and post-harvest levels to prevent viral contamination of the oysters. The most effective way to reduce health risks due to viruses in oysters is to implement risk management procedures to reduce the initial extent of contamination at the source (Pommepuy et al. 2004). Real-time RT-PCR methods can be used to identify sources of contamination and assess the effectiveness of intervention measures (Dore et al. 2010). The risk management should be site-specific. The first step is to carry out an extensive sanitary survey to identify and assess possible sites/sources of viral contamination. This should include intermittent contamination resulting from events/conditions such as untreated water effluent, storm-water runoff, sewer overflow, livestock slaughterhouse and processing effluent, commercial and recreational boat users, overflow from manure lagoons and seasonal influxes of people which could exceed the capacity of the available wastewater treatment system (e.g., influx of vacationers at beach resorts). Some of the common control measures used are discussed below along with their limitations.

18.3.1 Pre-harvest Control Measures

18.3.1.1 Prevention of Sewage Contamination of the Shellfish Cultivation Waters and Monitoring Environment

The majority of seafood-related illnesses are associated with contamination of the shellfish beds with human sewage. Safe shellfish production necessitates control of the discharge of untreated sewage into shellfish cultivation waters. The inappropriate dumping of excreta must be controlled, and may require enforcement of appropriate laws or regulations to dissuade people from this practice. A prior survey of oyster harvesting boats in the U.S. revealed that nearly 85% of the boats disposed of their sewage overboard (Kohn et al. 1995). Berg et al. (2000) reported oyster-associated outbreaks in Louisiana traced to overboard disposal of sewage. Similarly in New Zealand, recreational boats were linked to outbreaks of norovirus (Simmons et al. 2001). Shellfish harvesters should be educated about the risk associated with the practice of waste disposal into the water bodies.

Treatment of wastewater is often found to be inadequate in removing the entire viral load, with enteric viruses detected at considerable levels in water treatment plant effluent. The effectiveness of the wastewater treatment depends on the virus type, the initial load of the virus, and the retention time. Genotype I NoV strains are more resistant to treatment than genotype II, and thus are more often associated with shellfish related outbreaks (van der Berg et al. 2005; da Silva et al. 2007; La Rosa et al. 2007). NoV has been detected at levels ranging from none detected to 10^6 genome copies/L

of the treated sewage (van der Berg et al. 2005; da Silva et al. 2007; Iwai et al. 2009). On average, 10^3–10^4 genome copies/L were detected in treated sewage, and could increase 100- to 1000-fold during peak periods (Maalouf et al. 2010a).

In the U.S., tertiary treatment of wastewater typically involves chlorination prior to discharge. Such treatments provide some residual effect as compared to the use of UV treatment commonly used in the EU, which lacks any residual action and is also affected by the turbidity of the water. Adequate validation and subsequent verification of wastewater treatment effectiveness is needed to prevent discharge of inadequately treated water, particularly if discharged waters could impact oyster cultivation sites.

Shellfish cultivation or harvesting sites have traditionally been periodically examined for the presence of pathogenic enteric bacteria or indicators of fecal contamination such fecal coliforms and *E. coli*, with harvesting suspended when excessive levels of the bacteria are detected. However, it has been well established that such indicators have limited predictive value for viral pathogens such as NoV and HAV. Several alternative indicator microorganisms such as male-specific RNA (F-RNA) bacteriophages, somatic coliphages, bacteriophages infecting *Bacteroides fragilis*, and human pathogenic viruses have been proposed as potential indicators of viral contamination (Hernroth et al. 2002). The physical and genomic properties of F-RNA phages, frequently found in sewage and fecal contaminated waters, are similar to those of NoV and HAV (Dore et al. 2000, 2003) and are probably more representative of the human virus accumulation and survival kinetics in shellfish than enteric bacteria (Dore et al. 2003). These properties, together with the simplicity of enumeration, make F-RNA phage an attractive indicator organism for viral contamination of marine environments (Hernroth et al. 2002; Dore et al. 2003; Oliveira et al. 2011). F-RNA phages have some predictive capability for NoV, but only very weak predictive capability for HAV virus (Formiga-Cruz et al. 2003). The F-RNA phages are not specific for human viruses, and cannot differentiate between viruses of animal or human origin, thereby potentially over estimating the risk (Havelaar et al. 1986). A one-year study in The Netherlands showed the presence of phages in 67% of oyster samples, but there were no detection of pathogenic viruses such as NoV or HAV (Lodder-Verschoor et al. 2005). While rapid detection of viruses using genomic approaches have been improving, such testing is still not widespread and represents a significant barrier to effective pre-harvest control options.

18.3.1.2 Relaying and Depuration

Since, in many areas, the absence of pathogenic microorganisms including human viruses from oyster harvesting sites cannot be ensured to a high degree of confidence, shellfish cultivation and capture operations have traditionally employed two intervention techniques: relaying and depuration. The methods take advantage of the ability of the shellfish to purge microorganisms from their body under favorable conditions of temperature, pH, and salinity if provided an environment free of pathogens.

Depuration is the process of placing shellfish in tanks containing continuously disinfected water under controlled conditions. The depuration process usually takes 2–3 days to eliminate fecal indicator bacteria (Jaykus et al. 1994; Richards et al. 2010) but is less successful at virus removal, particularly if the water temperature is low. While depuration may result in complete elimination of bacteria in as little as 48 h, viruses are known to persist for up to 8 weeks in live shellfish. Traditional depuration cannot be relied on to remove viruses (Lees 2000; Greening et al. 2003; Nappier et al. 2008).

Relaying is the transfer of shellfish to clean natural waters that are approved for production (Sobsey and Jaykus 1991). Thus, it is the same approach as depuration but greater uncertainty in terms of the conditions that will foster virus elimination. It is generally done for an extended period of time, e.g., 10 days to 2 weeks (Richards et al. 2010). However, the major drawbacks to this intervention are the lack of acceptable sanitary shellfish growing waters, economic cost, and susceptibility to environmental

disturbances (e.g., temperature fluctuations, water movements, smothering/clogging by sediments, physiological stress, shell damage) often encountered during the relaying process (Richards 1988; Lees 2000). Furthermore, water quality of relaying areas is difficult to assure and there is the possibility of recontamination due to heavy rains and associated land runoff (Richards 1988; Lees 2000).

18.3.2 Post-harvest Control Measures

18.3.2.1 Heat Treatment/Cooking

As discussed above a substantial portion of oysters are consumed raw, and pre-harvest controls such as depuration or rinsing of the shellfish with potable water cannot ensure the absence of HAV or NoV. Therefore, consumers that wish to reduce the risk of these human viruses have to rely on some form of post-harvest processing. The most used method is cooking. Slomka and Appleton (1998) reported a 1.7-log reduction in feline caliciviruses (FCV) when cockles were immersed in boiling water for 0.5 min. This correlates with an internal temperature of 60 °C. At an internal temperature of 78 °C for 1 min, FCV (initially 4.5 log $TCID_{50}$/g present) could not be detected. For HAV, which is considered more heat resistant, internal temperatures of 85–90 °C for 1 min are needed for complete inactivation (Millard et al. 1987). Similarly, at an internal temperature of 88 °C (2 min) no infectious HAV (initially 10^5 infectious units) could be detected (Croci et al. 1999). The practice of heating until the shell opens is not considered adequate since the shellfish has an internal temperature of only 70 °C at this stage, which is insufficient to inactivate all the pathogenic viruses.

A limited number of oyster purveyors have introduced post-harvest technologies to reduce the levels of foodborne pathogens, particularly *Vibro parahaemolyticus* and *Vibrio vulnificus*. These intervention technologies include individual quick freezing (IQF) (see Sect. 18.3.2.4), Heat-Cool Pasteurization (HCP), and High Hydrostatic Pressure (see Sect. 18.3.2.3) (Hirneisen et al. 2010). However, these technologies are likely to have minimal impact on enteric viruses. A patented process of extended low temperature heating followed by rapid cooling is being used commercially to reduce *Vibrio* levels, the process would not be sufficient to inactivate NoV or HAV. Effective thermal inactivation of NoV can only be ensured with heat treatments such as baking, boiling at 100 °C for 30 min and roasting (Mormann et al. 2010).

The thermal inactivation of viruses in shellfish also depends on the food matrix. The nature of the shellfish tissue and concentration of viruses in the digestive diverticula hampers heat penetration. Variability in the size of shellfish, their viral content and cooking conditions make it difficult to set standards related to cooking times and temperatures. In their quantitative risk assessment of shellfish-borne HAV infections based on outbreak data, Pinto et al. (2009) estimated that attack rates were reduced by lightly cooking shellfish (i.e., until shells opened) and further reduced by cooking for an additional 5 min after the shells opened. However, they concluded that while cooking reduced the risk of HAV infections, cooking did not eliminate the risk.

18.3.2.2 Ionizing Radiation

A 95% (1.3 $\log_{10}$) reduction in HAV in shellfish was observed with a radiation dose of 3 kGy but led to changes in the organoleptic properties of the product (Mallet et al. 1991). Murine NoV 1 and human norovirus-like particles have been found to be resistant to gamma irradiation i.e., only a 1.7- to 2.4-log virus reduction on fresh produce was achieved with a dose of 5.6 kGy. The use of irradiation to eliminate the viral contaminants is generally considered impractical.

18.3.2.3 High Hydrostatic Pressure (HP)

High hydrostatic pressure (HP) has been used in the shellfish industry to inactivate *Vibrio* species without causing any change in the organoleptic quality of oysters (Berlin et al. 1999). A 3-log reduction of HAV was achieved by HP at 400 MPa in 1 min at 9 °C (Calci et al. 2005) and a 4-log reduction at 5 °C for murine NoV-1 (Kingsley et al. 2007). Chen et al. (2005) found that lower temperatures facilitated a 4- to 5-log reduction in FCV; at −10 °C at a pressure of 200 MPa (4 min) as compared to 0.3 log reduction at 20 °C. Similar findings were observed by Kingsley et al. (2007) who reported a 1.2-log reduction at 350 mPa (5 min) at 30 °C as compared to a 5.6-log reduction at 5 °C for MNV-1. HP is not effective against all viruses, e.g., poliovirus is quite resistant (600 MPa at 20 °C for 60 min) due to the presence of large capsid proteins (Grove et al. 2008; Wilkinson et al. 2001). Grove et al. (2009) studied HP inactivation of HAV in buffered growth medium containing salt (15 or 30 g/L) with pressures between 300 and 500 MPa for treatment times of 60 to 600 s. HAV was inactivated by > 3 log TCID50/ml after treatment with 500 MPa for 300 and 360 sec in medium containing salt at 15 and 30 g/L, respectively. The development of strains resistant to HP has been hypothesized, but needs further study (Smiddy et al. 2006). Thus, HP has emerged as a potential treatment to inactivate viruses in shellfish. Commercial processors use around ~275–300 MPa of pressure for up to 3 min, thus maintaining the taste, juiciness and texture of raw product. The treated products have a slightly cooked appearance (EFSA 2012).

18.3.2.4 Refrigeration and Freezing

Control of bacterial pathogens by at low temperature to minimize or prevent their growth is a widely accepted practice. Further, mild heating followed by rapid refrigeration does reduces the levels of *Vibrio* spp. (Anon 2017). However, such treatments are not useful for viral pathogens; viruses do not multiply in foods and low temperatures (refrigeration/freezing) facilitate their preservation (Lees 2000; Papafragkou et al. 2006).

18.3.2.5 Post-harvest Handling and Hand Washing Practices

Though post-harvest contamination of shellfish is not a major route of transmission, preventive measures should be taken to avoid accidental contamination. After harvesting, all processing operations should be performed in accordance with a HACCP plan. Shucking, packaging, transport, *etc.* should be done under hygienic conditions. Live and shucked shell-stock should be maintained at 10 °C and ≤7.2 °C, respectively. Food contact surfaces should be thoroughly cleaned with potable water and chlorine-based disinfectants (NSSP 2007). Water used for cleaning and making ice should be of potable quality or can be treated with UV light.

Various studies have been conducted on the effectiveness of ethanol as a disinfectant for NoV and its surrogate viruses, FCV and MuNoV-1. Ethanol has been found to be ineffective in completely inactivating NoV. Reductions of < 2 $\log_{10}$ after a contact time of 8 min were found in one study (Duizer et al. 2004), while other workers (Park et al. 2010) reported a 0.5-$\log_{10}$ (Liu et al. 2010) and 2.2-$\log_{10}$ reduction after contact time of 30 sec and 5 min, respectively, at various ethanol concentrations. Studies carried out at long-term health care facilities have demonstrated the ineffectiveness of alcohol-based hand sanitizers (ABHS) in controlling the spread of NoV, with the incidence of NoV being 6 times higher in facilities that use ABHS for hand sanitization as compared to the facilities using conventional hand washing (Blaney et al. 2011). Whitehead and McCue (2010) reported that a 60% ethanol and quaternary ammonium compound spray having a pH of 10.8 completely inactivated FCV in 1 min. The use of hand-washing water at a minimum of 43 °C is recommended by the NSSP in all shellfish handling facilities (NSSP 2007).

Appropriate tagging should be done for traceability of the shell-stock in case of any outbreak.

18.3.3 Vaccination Control of HAV

Vaccines against HAV infections are highly efficacious and provide long-lasting protection in adults and in children above 1–2 years of age (http://www.who.int/vaccines/en/hepatitisa.shtml). Hepatitis A infection rates in the U.S. have declined by 92% since hepatitis A vaccines became available in 1995. The U.S. CDC recommends HAV vaccination for all children at 1 year of age, for people who are i) at increased risk for infection, ii) at increased risk for complications from hepatitis A, and iii) for anyone wishing to obtain immunity (http://www.cdc.gov/hepatitis/HAV/HAVfaq.htm).

18.3.4 Education, Risk Communication

Risk communication and education play an important role for food safety systems in the delivery and exchange of information along the farm to table continuum. All personnel involved in the process have to be aware of their role and responsibility in protecting food from contamination; food hygiene training is very important. The role of consumers in the prevention of foodborne illness is also substantial, and consumers should be made aware of the potential health risks associated with eating raw shellfish, and that shellfish-borne viral infections can be prevented by cooking thoroughly and avoiding cross-contamination after cooking. Food safety public health campaigns such as the U.S. national public campaign Fight BAC® (FIGHT BAC® 2009) and the New Zealand national public Food Safe Partnership campaign (NZFSA 2006), are good sources of information. Raw shellfish are included in the U.S. FDA 2009 Code requirement which allows food service establishments to serve, at the customer's request raw or undercooked foods as long as the customers is informed of the risks associated with consuming such foods and the customer is not part of a high-risk group. The warning, "Consuming raw or undercooked meats, poultry, seafood, shellfish, or eggs may increase your risk of foodborne illness," is prominently displayed on U.S. menus.

For clinicians and laboratorians, prompt recognition of infection in patients who seek medical attention for illness after eating raw or undercooked seafood is important for appropriate testing and early treatment. Rapid reporting of cases to public health authorities is critical to identify both contaminated seafood and risky harvest areas, allowing prompt implementation of control measures and decreasing the impact of potential outbreaks.

18.3.5 Acceptable Levels of Consumer Protection/Establishing ALOP/FSO/PO

Establishing an ALOP/FSO for NoV or HAV in oysters is complicated by the fact that the ID_{50} is not known with certainty, that current environmental and pre-harvest controls have limited effectiveness, that post-harvest interventions do not greatly reduce exposure, and that most servings are consumed without cooking. Since human viruses do not multiply in shellfish, this would be a situation where the FSO and PO for the intact oyster would be equivalent.

Raw oyster consumers, a significant proportion of the total population of consumers, often consume multiple oysters in a meal. Assuming a serving size of 360 g (approximately the weight of six shucked oysters), a concentration of 1 genome copy/serving would be 0.0028 genome copies/g which is equivalent to < -2.56 $\log_{10}$ genome copies/g. At this level of contamination, the risk of illness is 6.9 x 10^{-7} per meal, or one in 1.4 million servings of six oysters. However, from the available published information (as discussed earlier) the level of contamination of oysters with NoV is often higher than one per serving.

Establishment of an ALOP/FSO for enteric viruses presents a challenge, particularly for NoV. EFSA (2012) concluded that the consumption of raw oysters is not likely to be compliant with an FSO-based approach at the current time. Instead, public health goals will likely have to be met by limiting the number of individuals who consume raw oysters through warning labels/education programs, and minimizing contamination of shellfish cultivation areas with human waste. For HAV, this could potentially change if effective vaccination programs continued to expand to levels where immunization is almost universal.

The establishment of an ALOP/FSO/PO is likely to have more utility for cooked oysters and related molluscan shellfish products. No single prevention or intervention strategy is able to eliminate reliably the risk of NoV or HAV infections associated with shellfish to an acceptable level. However, a combination of strategies may provide sufficient stringency to achieve an acceptable level of consumer protection. For example, the level of thermal processing of canned oyster stews and soups is sufficient to reduce the risk of HAV to acceptably low levels. Pinto et al. (2009) concluded in their risk assessment that while cooking did not eliminate the risk of HAV, it did decrease the risk compared to raw shellfish, with cooking to a well-done state being more effective. A difficulty in considering this approach currently is a lack of relevant data on the degree of control needed along the food chain to achieve an appropriate level of control, and the risk management controls and metrics to implement such an integrated system. In particular, gathering information about the level of viral contamination in the shellfish cultivation environment in a timely manner is both a technical and an economic barrier, especially if environmental conditions can change rapidly. The bio-variability and uncertainty currently associated with the various factors that affect the risk of infection have not been sufficiently and rigorously established to design a food safety system that enables the cultivation and processing of oysters to be sufficiently managed to achieve an appropriate level of stringency with a high degree of confidence. Even if such data were available, and enabled an appropriate risk management strategy, taking such an approach for live oysters destined for cooking in the home or in restaurants could be a major challenge in terms of assuring that the preparer actually cooks the oyster to the degree specified for achieving the target level of control.

18.3.6 Identifying and Establishing Performance Objectives/Performance Criteria

A performance criterion is the required outcome of one or more control measures at a step or combination of steps to achieve an FSO (see Chap. 3). Performance criteria are usually applied at steps where hazards can either be reduced or where hazards may increase.

To arrive at a performance criterion for the control measures needed to meet an FSO for viruses in oysters, the ICMSF conceptual model, Eq. 3.1, can be used:

$$H_o - \Sigma R + \Sigma I \leq FSO$$

where:

FSO = Food safety objective
H_o = Initial level of the hazard
ΣR = Total (cumulative) reduction of the hazard
ΣI = Total (cumulative) increase of the hazard

FSO, H_o, R, and I are expressed in $\log_{10}$ units, R is negative (reduction) and I positive (increase).

For this approach to be useful, it is necessary to decide an ALOP that can be translated into the FSO, and from which Performance Objectives and Performance Criteria can be derived. However, there is no generally accepted ALOP for NoV infection from foods. In the absence of such an ALOP, we provide here an example based on the current rate of food-borne infections from all foods in developed nations which is, approximately, 1 per 10,000 meals (Scallan et al. 2011; Kirk et al. 2014). However, the status quo for illnesses per meal risk is for *all* food-borne pathogens. Accordingly, for the purposes of illustration we will adopt a risk of illness of NoV of 1 per 1 million servings of bivalve shellfish, assuming that a serving is 360 g, and that the ID_{50} for *illness* from NoV is 1 million infectious particles. If all shellfish were uniformly contaminated, this ALOP translates into:

$$H_o - \Sigma R + \Sigma I \le -2.10 \log_{10} / \mathrm{g} \left(\text{disagreggated particles} \right)$$

Since growth is not possible in bivalve molluscs, and since recontamination is unlikely, the equations can be simplified to:

$$H_o - \Sigma R \le -2.10 \log_{10} / \mathrm{g}$$

18.3.6.1 Controlling Initial Levels in Raw Materials

Controlling the initial levels of norovirus in shellfish is the most important control measure, and can be achieved by production of shellfish in clean water. Traditionally, shellfish harvesting areas are periodically monitored for contamination by pathogenic enteric bacteria such as *Salmonella*, microbiological indicators of fecal contamination, or pathogenic *Vibrio*, with harvesting suspended if the levels exceeded specified limits. However, monitoring for viral contamination may not be controlled by this activity because of the poor correlation between bacterial indicators and NoV and HAV. This also reflects the fact that even tertiary treatment of wastewater has little or no effect on virus populations. It is feasible to monitor environmental waters or shellfish for viral genomes but this has not been widely adopted as a preventive measure, but is used in subsequent outbreak investigations. There are predictable periods of risk of elevated levels of viruses (e.g., after excess rainfall leads to sewage treatment bypasses) that can be identified by reviewing the records of contamination. Such events can then be used to increase the extent of monitoring of cultivation areas, and to prevent harvesting during such high risk periods or after accidental contamination from unexpected sources. This is becoming increasingly feasible both technically and economically with the development of new environmental sensing technologies and testing methodologies.

18.3.6.2 Prevention of Re-contamination After Harvest and Before Cooking

Appropriate personal hygiene practices are very important to prevent norovirus transmission. Proper hand washing with soap and running water for at least 20 s is the most effective way to reduce norovirus contamination on the hands (CDC 2017c). Considering the highly infectious nature of NoV and HAV and the importance of person-to-person transmission in their epidemiology, exclusion of infected persons from the food chain is advised for a period of time during and after active signs of an infection is considered a practical means of interrupting transmission of virus. However, the effectiveness of this control may be limited due to shedding of the virus before and after overt symptoms and the possibility of asymptomatic carriers.

18.3.6.3 Reducing NoV Levels by HHP

Infectious virus titers can be reduced by up to 1000-fold using commercial high pressure processing at 275–300 MPa of pressure for 3 min. Thus, H_o values of ≤ 0.90 $\log_{10}$/gram (~8 genome copies gram) could potentially be tolerated if HHP was applied reliably, provided that strategies to control and monitor H_0 were also implemented.

18.3.6.4 Reducing HAV Levels by Cooking

Heat treatment/cooking is an important post-harvest step to reduce virus levels in molluscs and thus reduce the risk of foodborne infection, even though such treatments may not guarantee total inactivation of viruses. Using HAV as an example, cooking at low temperatures until the shell opens has been estimated to produce a 99.46% reduction in HAV titers, whereas cooking to an internal temperature of 90 °C for 90 s (cooking for 5 min after opening of shell) reduces HAV levels by 99.86% (Pinto et al. 2009). Thus, H_o values of ≤ 0.48 $\log_{10}$ genome copies/g (~3 HAV genome copies/g) would be expected to be reduced to ≤ -1.79 $\log_{10}$ genome copies/g if gentle cooking were applied reliably. Similarly, an H_o value of ≤ 1.07 $\log_{10}$ genome copies/g (~12 HAV genome copies/g) would produce an equivalent reduction to ≤ -1.79 $\log_{10}$ genome copies/g if cooking to an internal temperature of 90 °C for 90 s were applied reliably.

18.3.6.5 Prevention of Re-contamination After Cooking

Personal hygiene practices of no bare-hand contact with ready-to-eat foods is recommended.

18.3.7 Acceptance Criteria

18.3.7.1 Organoleptic Criteria

The presence of viruses in shellfish cannot be assessed by organoleptic evaluation and, thus, organoleptic criteria are not applicable.

18.3.7.2 Chemical and Physical Criteria

No physical and /or chemical criteria are applicable to assess the presence of NoV and HAV in shellfish.

18.3.7.3 Microbiological Criteria

Currently available virus detection methods are not adequate to establish microbiological criteria for viruses in molluscs. The current limit of detection for NoV from oysters is 20 RNA genome copies/2 g homogenate (pooled sample of 10 oysters) and current limit of quantitation for NoV from oysters is 100 RNA genome copies/2 g homogenate (EFSA 2012). Further, presence/absence sampling affects the results due to the potential for overestimating risk with pooled samples and the potential for false negative results when individual oyster testing. The presence of infectious *vs*. non-infectious viral particles also needs to be considered when setting microbiological criteria.

18.4 Relating Risk Management Metrics to Stringency of Food Safety System

18.4.1 HACCP Plans

The NSSP-guide to the control of molluscan shellfish (NSSP 2007) describes the measures that are currently recommended to ensure safe production of shellfish.

18.4.2 GHP Programs

Proposed Codex guidelines (Codex 2012) on 'The Application of General Principles of Food Hygiene to the Control of Viruses in Food' provide advice to governments for the control of human enteric viruses in food, especially NoV and HAV, with a view towards protecting the health of consumers and ensuring fair practices in food trade. The guideline also contains an annex on specific measures for the control of HAV and NoV in bivalve molluscs.

18.4.3 Regulatory Requirements and Criteria

There is presently no internationally harmonized approach to shellfish testing for human viruses. While long recognized that the use of bacterial indicator microorganisms is a poor predictor of contamination of bivalve molluscs with human viruses, these indicator bacteria continue to be the primary means for assessing growing waters and shellfish. For example, in the U.S. the standards for molluscs are centered on the detection of coliforms and *Escherichia coli* in the shellfish growing waters. In EU, standards focus on the number of microorganisms/100 g of shellfish flesh (EURL 2014). In global food trade, it is generally recognized that additional microbiological guidance, including the potential establishment of microbiological criteria, are needed to improve the virological safety of bivalve shellfish.

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Chapter 19
Campylobacter in Chicken Meat

19.1 Introduction

The global trade in chicken meat is extensive. In 2016 world-wide imports totalled 8.9 million tons and world-wide exports totalled 10.8 million tons. Brazil is the largest exporter of chicken meat in the world at 4.1 million ton per annum in 2016 (USDA 2016). Production of chicken meat starts at the hatchery where chicks are reared. These are stocked into chicken farms where they are grown to the required weight. Chicken production farms range from organic operations which are committed to "free range rearing to intensive, enclosed chicken houses where birds are ready for slaughter at around 5–7 weeks of age. The ensuing processing stages of slaughter, dressing and chilling are highly mechanized with line processing speeds approaching 175 birds per min. This is followed by distribution and retail sale under chill or frozen conditions. There are many opportunities during primary production and processing for chickens and chicken meat to become infected with pathogenic bacteria. *Salmonella* and *Campylobacter* are the principle pathogens of concern, with the latter being the focus of this chapter.

Thermotolerant *Campylobacter* are the leading cause of bacterial enteric human infections in many countries. Sources of infection can be the environment, water, animal contact and food. Estimates of the percentage of cases that are attributable to food range from 42% to 80% (ESR 2011). A high proportion of these cases are sporadic and linked with the handling, preparation or consumption of poultry meat. In the European Union (EU) it is estimated that this infection route accounts for 20–30% of human cases of campylobacteriosis, while 50–80% may be attributed to the chicken reservoir as a whole (EFSA 2010a).

The aim of this chapter is to illustrate how the principles for setting a food safety objective (FSO), associated performance objectives (POs) and where appropriate microbiological criteria (MCs) can be applied to the control of *Campylobacter* contamination of raw chicken meat during production and processing as well as preparation and consumption in the home. It is not intended that this chapter is a comprehensive review of available data or previous work in this area. However, sufficient information will be provided to put the problem into context and demonstrate the steps necessary for the application of these risk management metrics as they relate to the risk posed by *Campylobacter* on raw poultry.

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19.2 Risk Evaluation

19.2.1 Hazard Identification

Campylobacter are non-spore forming, gram negative spiral-rod shaped bacteria. They are motile with a corkscrew-like motion by means of a polar flagellum. *Campylobacter* do not ferment or oxidize carbohydrates and obtain their energy from amino acid metabolism or intermediate compounds formed by the tricarboxylic acid cycle. There are around 20 species in the genus although this is still debated in the literature (Silva et al. 2011).

In the context of poultry contamination the thermophilic species pose the greatest risk. Thermophilic *Campylobacter* have an optimum growth temperature of 41.5 °C and grow best between 37 °C and 45 °C. They do not grow below 30 °C. Thermophilic *Campylobacter* grow best in moist environments and display optimum growth at a water activity of 0.997 with a lower limit of 0.987. Their optimal pH range is between pH 6.5 and 7.5 and they do not grow above pH 9.0 or below pH 4.9. They are sensitive to oxygen and oxidizing radicals and are classed as microaerophilic; they grow best in an atmosphere containing 10% carbon dioxide and 5–6% oxygen (Silva et al. 2011).

Campylobacter has been the most common bacterial cause of gastroenteritis in Europe since 2005. In 2014 the incidence rate for confirmed cases was 71 cases per 100,000 population (EFSA 2015). In the United States the 2013 incidence rate for *Campylobacter* was 13.8 cases per 100,000 population based on FOODNET data from 10 U.S. sites (CDC 2013). Higher infection rates are seen in Australia and New Zealand. In Australia the crude incidence rate for *Campylobacter* infection was 113 cases per 100,000 in 2010 (Australian Government 2012). New Zealand reported a *Campylobacter* notification rate of 168.2 in 2010 (ESR 2011). However, under-reporting rates of between 10–100 times have been suggested (EFSA 2010a) which means that the burden of illness on the population is considerably larger than these figures suggest.

The main reservoir for *Campylobacter* are wild and domesticated birds and mammals. The main food animal reservoirs are cattle, pigs, poultry and sheep (Moreno et al. 1993; Chuma et al. 2000; Oporto et al. 2007). *Campylobacter* can survive in moist environments and water courses for up to 3 months (EFSA 2011). The optimum growth temperature of these species makes them ideally suited to colonization and growth in the alimentary tract of chickens where normal body temperature is between 40.5 °C and 42.5 °C. Infections associated with the consumption of poultry are caused principally by the species *C. jejuni,* followed by *C. coli* and, to a much lesser extent, *C. lari*.

19.2.2 Hazard Characterization

19.2.2.1 Disease Manifestation

Campylobacter causes campylobacteriosis in humans, a leading cause of bacterial gastrointestinal illness in many countries (Scanlon et al. 2013). At present, *C. jejuni* and *C. coli* are the main causes of campylobacteriosis in humans. *C. jejuni* infection causes enteritis in humans. Common manifestations include watery diarrhea or bloody diarrhea with fever, abdominal cramps and nausea. Incubation period ranges 1–10 days but typically are between 2 and 5 days. Symptoms typically last 1 day to 1 week or longer (usually 5 days). Excretion of the microorganism in stools occurs on average for 2–3 weeks and is generally self-limiting. The maximum attack rate is 45% (ESR 2007). *C. coli* enteritis is similar to *C. jejuni* enteritis.

Campylobacter jejuni is also associated with systemic infections, such as bacteremia and the post-infectious complications such as Guillain–Barré Syndrome (GBS). GBS is rare with an incidence of

<1 per 1000 infections and is an acute demyelinating disease of the peripheral nervous system that affects 1–2 persons per 100,000 population in the United States each year. The risk of developing GBS is increased after infection with certain *Campylobacter* serotypes (Allos 2001). GBS can occur 1–3 weeks after gastroenteritis and approximately 20% of persons with GBS are left with some form of disability and approximately 5% die (ESR 2007).

Local complications of *Campylobacter* infections can occur as a result of direct spread from the gastrointestinal tract and can include cholecystitis, pancreatitis, peritonitis, and massive gastrointestinal hemorrhage. Extraintestinal manifestations of *Campylobacter* infection are quite rare and may include meningitis, endocarditis, septic arthritis, osteomyelitis, and neonatal sepsis. Bacteremia is detected in <1% of patients with *Campylobacter* enteritis and is most likely to occur in patients who are immunocompromised or among the very young (infants less than 1 year old), young adults in their twenties with the incidence higher in males (up to 45 years of age) (Allos 2001; ESR 2007). Reiter's syndrome (reactive arthritis) has been associated with *Campylobacter* as well at approximately 1% incidence of all campylobacteriosis cases, although other bacteria causing foodborne illness may also have the same effect (ESR 2007; Pope et al. 2007).

19.2.2.2 Antimicrobial Resistance

Numerous studies on antimicrobial resistance of *C. jejuni* and *C. coli* species have shown resistance to a variety of antimicrobial agents with the highest number of isolates resistant to ciprofloxacin, tetracycline, nalidixic acid, amoxicillin and colistin (Wieczorek et al. 2013; Jamali et al. 2015). Lower percentages of isolates (between 2.5% and 9.9%) were resistant to streptomycin, neomycin, erythromycin, chloramphenicol and ampicillin, but not to gentamicin. However, other studies have shown limited resistance to gentamicin (Wieczorek et al. 2013). Some isolates are also multi-resistant i.e. to two or more antibiotics (Jamali et al. 2015). Other antimicrobial agents against which both *C. jejuni* and *C. coli* have shown resistance are aztreonam, sulfamethoxazole, trimethoprim, and other quinolones (norfloxacin, ofloxacin, levofloxacin) and fosfomycin (Yabe et al. 2010). Furthermore, there is an increasing resistance to the fluoroquinolones (ciproflaxin) in particular, as shown by Yabe et al. (2010) when results were compared between the years 1996–2001 and 2007–2009.

19.2.2.3 Virulence Factors

Flagella-mediated motility (determined by the *flaA* and *flhA* genes), which is associated with adherence, invasion and colonization, adherence to intestinal epithelial cells (cadF and docA gene products), invasion and survival in the host cells (ciaB, iam, wlaN, and virB11 markers) as well as the ability to produce toxins, particularly cytolethal distending toxins (*cdt* genes) are important virulence factors involved in campylobacteriosis (Bang et al. 2003; Datta et al. 2003; Fernandes et al. 2010).

19.2.2.4 Dose-Response Models

The probability of campylobacteriosis is driven by the conditional probability of ingesting a dose of *Campylobacter*, the conditional probability that these organisms will survive passage through the stomach and the conditional probability of illness developing once a person is infected. Infection is modulated by the survival characteristics of the microorganism and its virulence characteristics. Host factors also contribute to the probability of infection and illness, including stomach contents, general health status and immune system status.

An excellent discussion on *Campylobacter* dose-response can be found in the FAO/WHO risk assessment on *Campylobacter* in chickens (FAO/WHO 2009). The subject will only be discussed briefly here and is based on this information. Dose response modelling is the application of a mathematical expression to describe the probability of an individual being infected given the ingestion of a known dose of pathogenic bacteria. The underlying data can result from epidemiological studies or more commonly, feeding trials with animals or ideally, humans. If epidemiological data are to be used for dose response modelling then it is essential that the attack rate and the ingested dose is available. The latter is rarely collated since elucidating the infectious dose is not the primary aim of epidemiological investigations. Nevertheless a dose-response relationship between consumption of liver pâté and infection with *Campylobacter* from a U.K. outbreak has been reported (Edwards et al. 2014) although these data do not allow for modeling. Feeding trial data can also have its limitations as the administered doses are usually high in comparison to the true number of organisms ingested from food. Furthermore, feeding trial volunteers are limited to healthy individuals and are not representative of the population as a whole. It is also difficult for such trails to replicate the range of virulent organisms that typically make up the population of pathogens.

For *Campylobacter*, only feeding trial data are available and these data are from a single trial (Black et al. 1988). They used two strains of *C. jejuni* that had been associated with human illness, administered in milk to healthy volunteers in the USA. Approaches to modeling these data involve the assumptions that either there is a threshold dose below which there is no infection or alternatively that a single *Campylobacter* cell has a probability of causing illness and that this probability increases as the number of microorganisms increase. The latter model type has been applied to describe the *Campylobacter* dose-response relationship.

A Beta-Poisson model was applied by the FAO/WHO (2009) and is described in detail in that reference. The resulting probability of infection can be estimated from Eq. 19.1.

$$P_{\text{inf}} = 1 - \left(1 + \text{N} / \beta\right)^{-\alpha} \tag{19.1}$$

Where N is the dose ingested (cfu) and the dose-response parameters $\beta = 59.95$ and $\alpha = 0.21$.

The probability of illness with a given dose could not be calculated from the feeding trail data as there was no clear relationship to the model. However, the pooled data showed that 29 people became ill of the 89 people infected (33%) (FAO/WHO 2009).

19.2.3 Exposure Assessment

Campylobacteriosis resulting from the consumption of chicken meat starts at primary production with the infection of chickens with *Campylobacter*. Infection with *Campylobacter* has been reported to result in no production loss or adverse symptoms in poultry (Newell and Fearley 2003). However, other studies have reported increased mortality in young birds, and an association between infection with avian pathogenic *Escherichia coli* and presence of *Campylobacter* (Neill et al. 1984) and also associations between *Campylobacter* and leg and feet lesions (Bull et al. 2008). Unlike *Salmonella*, vertical transmission of *Campylobacter* from parent and grandparent stock is not considered to be a source of infection (EFSA 2011). However, a systematic review concluded that vertical transmission occurred, but its relative importance could not be established compared to other sources of infection (Agunos et al. 2014). Reports suggest that slower-growing birds have better inherent gut health and are less likely to carry *Campylobacter,* whereas fast-growing birds have severe diarrhoea, with damage to the ileum and are more prone to pododermatitis and hock marks (Williams et al. 2013). This is clearly an area requiring further work.

19.2.3.1 On-Farm Biosecurity and Infection of Chickens

The main focus of research into the control of *Campylobacter* on-farm has been prevention/reduction of horizontal transmission. Cattle, sheep and wild birds are associated with asymptomatic shedding of *Campylobacter* and may act as environmental reservoirs for poultry farms (Ellis-Iversen et al. 2012). Cattle are reported to be the primary non-poultry reservoir (Agunos et al. 2014).

Contamination of chickens, early in the production cycle, is related to the ingress of *Campylobacter* from the environment. Once introduced, shedding of *Campylobacter* in the birds' feces leads to colonization of the flock within a few days (Bull et al. 2006). Consequently, effective biosecurity on-farm is essential. Apart from colonization of other animals, *Campylobacter* has been found in the farm environment outside the poultry houses in or on puddles, water sources and farm equipment. In poultry sheds, *Campylobacter* has been found on anteroom surfaces, in drinkers and in the sheds between production cycles (Agunos et al. 2014). Pests can also play a significant role in the introduction of *Campylobacter* into poultry houses; flies in particular have been identified as a concern during the spring and summer (Hald et al. 2004). Catching and transport equipment are also a source of *Campylobacter* including the vehicles, catchers and in particular the crates and modules that are used to transport the chickens to the slaughter house (Agunos et al. 2014). In this respect the practice of thinning, where partial depopulation of flocks is undertaken, has been identified as a particular risk regarding the introduction of *Campylobacter* into poultry houses (Allen et al. 2008; EFSA 2011; Koolman et al. 2014). Other factors that affect the prevalence and numbers of *Campylobacter* on chickens are age at slaughter and season. An analysis of the relative importance of primary production factors that affect *Campylobacter* infection of flocks listed, in order of importance, "age of chicken house, rodent control, age of chicken at introduction of whole wheat in the chicken feed, age of chicken at slaughter, storage of whole wheat, number of chimneys on the chicken house, having one vs. more chicken houses on the farm, and location of the chicken farm in relation to cattle density" (Sommer et al. 2013).

Data collected from various studies in a number of countries have shown that a large proportion of chicken flocks are colonized with *Campylobacter* at slaughter. For example, in 2001 a study in the USA found 28 of 32 of flocks (87.5%) were positive for *Campylobacter* (Stern et al. 2001). A UK study examined 789 chicken batches originating from 214 farms and found an average *Campylobacter* prevalence of 35% in non-thinned flocks (Bull et al. 2008). Another UK study on 1174 mixed thinned and non-thinned chicken batches presented at slaughter found on average, 79.2% prevalence (Lawes et al. 2012). In Japan, 67 of 142 flocks (47.2%) were found to be infected with *Campylobacter* (Haruna et al. 2012). Similar prevalence of *Campylobacter* was found in French chicken flocks at slaughter, 77.2% (95% CI: 73.2; 81.2) with an average count in the cecal contents of 8.04 log cfu/g (range 4.2–10.6 log cfu/g) (Hue et al. 2010). A comprehensive European baseline study on *Campylobacter* in chickens was conducted by EFSA in 2008. Sampling of 10,132 chicken batches from 561 slaughterhouses in 26 countries across the EU found a prevalence of 72.1% (95% CI: 68.5; 73.7) (EFSA 2010b).

19.2.3.2 Chicken Slaughter

Studies of *Campylobacter* contamination of carcasses during slaughter have identified a number of critical steps. Guerin et al. (2010) conducted a systematic review that looked at the results from 32 articles published in the scientific literature. They found that the "scalding" stage where feathers are loosened by immersion of dead chickens in hot water, resulted in a decrease in *Campylobacter* prevalence and concentration on carcasses. The higher the temperature of the scald tank, i.e., 58 °C compared to 55.4 °C, the greater the decrease in prevalence. Duffy et al. (2014) conducted a quantitative through chain analysis on 4 flocks processed at 2 slaughter plants in Australia (2 flocks each plant). They found that the scalding stage (55 °C /2 min 20 s or 2 min 30 s) resulted in average reductions

ranging from 1.54 log cfu/carcass (sd.0.29 log cfu/carcass) to 1.99 log cfu/carcass (sd. 0.37 cfu/carcass). Presence of *Campylobacter* in scald water has been reported (Stern et al. 2001).

The next significant step is defeathering where mechanical plucking 'fingers' remove the loose feathers. This step was found to result in an increase in prevalence and concentration. Guerin et al. (2010) suggested the possible reason being escape of fecal material through the cloaca by the action of the picker fingers pressing on the abdomen. The evisceration process resulted in an increase in prevalence but a decrease in numbers although the reason for the latter effect was not clear and data was limited. In contrast, Duffy et al. (2014) found no significant difference in counts before and after the defeathering/evisceration step for any of the 4 flocks monitored.

It is common in poultry slaughter plants to use washers to remove blood and fecal contamination on chicken carcasses after evisceration. Some facilities add chlorine at levels around 50 ppm free chlorine while others do not. It is unlikely that added chlorine at this step would affect reductions in *Campylobacter* although if water is recycled it could reduce the probability of cross contamination (Codex Alimentarius Commission 2011). However, the presence of *Campylobacter* in chill/recycled water used in processing facilities has been reported by Stern et al. (2001). Guerin et al. (2010) concluded that the carcass washing step had a variable effect on prevalence with studies showing decreases, increases and no change in prevalence. However, concentration of *Campylobacter* generally decreased. Few quantitative studies have been reported under real processing conditions. In one such study, Bashor et al. (2004) quantified the effect of inside/outside bird washers on natural *Campylobacter* contamination on carcasses in 4 poultry slaughter plants in the USA. Each plant used between 2 and 3 washers in sequence although studies showed that the main reduction was achieved after the first washer. Reductions in mean *Campylobacter* concentrations ranged between 0.26 and 0.63 $\log_{10}$ cfu/carcass.

The effect of antimicrobial interventions was also studied *in situ* by Bashor et al. (2004). They reported that a trisodium phosphate spray (12% solution, pH 11, 15 s contact time) resulted in a reduction in *Campylobacter* of 1.03 $\log_{10}$ cfu/carcass and an acidified sodium chlorite spray (1200 ppm, pH 2.5. 15 s contact) resulted in a reduction in *Campylobacter* of 1.26 $\log_{10}$ cfu/carcass.

Following the washing step the next process used in poultry slaughter plants around the world is chilling. This is achieved by either emersion chilling in water (with chlorine or other antimicrobials) or air chilling. The antimicrobials used in emersion chilling are not considered to have a significant effect on *Campylobacter* reduction over and above the physical action of the water; however, they do reduce the probability of cross contamination (FAO/WHO 2008). In their review, Guerin et al. (2010) reported that the decrease in *Campylobacter* levels from emersion chilling ranged between 0.8 and 1.7 $\log_{10}$ cfu/carcass. Duffy et al. (2014) showed average decreases of 3.28 (sd. 0.47), 1.02 (sd. 0.3), 4.12 (sd. 0.25) and 3.59 (sd. 0.3) log cfu/carcass for emersion chilled carcasses with water temperatures/times/ free available chlorine levels of 4.2 °C/30 min/ 3.5 ppm, 6.9 °C/45 min/ 1 ppm, 4.4 °C/30 min/ 2.7 ppm, 7.0 °C/45 min/ 1.0 ppm respectively. There was no correlation between *Campylobacter* inactivation levels and chill temperature or chlorine concentration. Guerin et al. (2010) also noted that water emersion chilling appeared to result in a greater decrease in *Campylobacter* on carcasses post chill than air chilling although the authors noted that there were only a few studies on which to judge this difference. They reported that only 2 studies reported pre- and post-air chilling reductions that ranged between 0.2 $\log_{10}$ cfu/g and 0.4 $\log_{10}$ cfu/g.

The variation in *Campylobacter* contamination on chicken at the end of slaughter can be seen from a large international study. A baseline study of *Campylobacter* levels on chicken skin at the end of slaughter (n = 9200) found significant variation among EU member states (EFSA 2010b). For the EU as a whole, the percentage of samples below 10, 10–99, 100–999, 1000–10,000 and above 10,000 cfu/g skin were 46.6%, 12.5%, 19.3%, 15.8% and 5.8%, respectively. These data suggest a mean log-count of 1.62 $\log_{10}$ cfu/g skin (Sd: 2.32 $\log_{10}$ cfu/g skin[1]).

[1] "Intervals recorded as <10 cfu/g were set at 1 cfu/g and >10,000 cfu/g were set at 50,000 cfu/g before $\log_{10}$ transformation. The mid-point value of the interval was used for the X, P proportions of the cumulative data which were fitting to a normal distribution using @Risk™."

19.2.3.3 Retail Storage and Distribution

Campylobacter survives on chicken meat during chill and frozen storage through the retail distribution chain. *Campylobacter* counts on naturally contaminated chicken carcasses were not significantly different after 14 day storage at 2 °C packed in air and a variety of modified atmosphere packages (Byrd et al. 2011). Only storage in 100% O_2 showed a small reduction (0.15 $\log_{10}$ cfu/carcass). However, in another study *Campylobacter* inoculated onto chicken carcasses declined by 1.88 and 2.33 $\log_{10}$ cfu/g during chill (7 d, 4 °C) and frozen (28d, −20 °C) storage in air, respectively (Maziero and de Oliveira 2010). Smaller average reductions of *Campylobacter* on inoculated chicken fillets were reported as 0.52 $\log_{10}$ cfu/cm^2 after 7 days and 1.07 $\log_{10}$ cfu/cm^2 after 14 days storage at 2 °C in air (Meredith et al. 2014). There was no further significant reduction up to the 17 days storage at 2 °C in air and a range of other gas flush options were also evaluated (Meredith et al. 2014). The authors noted that the presence of oxygen was associated with the largest reductions in *Campylobacter* numbers during chill storage (Meredith et al. 2014). However, in a trial of frozen and chill storage there was no significant reduction *Campylobacter* in naturally contaminated chicken carcasses during storage at 3 °C for 7 days (Georgsson et al. 2006). However, frozen storage over 31 days at -20 °C achieved a reduction in *Campylobacter* on chickens from 5 flocks of 0.65 to 2.87 $\log_{10}$ cfu/1000 g chicken (Georgsson et al. 2006) with an average reduction across the five flocks of 1.77 $\log_{10}$ cfu/1000 g chicken (sd = 0.79 $\log_{10}$ cfu/1000 g chicken). In summary, it would appear that any reduction in *Campylobacter* during chill storage in the retail distribution chain is inconsistent with differences between results achieved with inoculated and naturally contaminated chicken meat. Reductions are also affected by packaging gas type with better reductions in an oxygen rich atmosphere. Frozen storage appears to result in reduction of *Campylobacter* numbers on chicken meat (Byrd et al. 2011).

The variation in *Campylobacter* contamination on chicken at the end of retail can be seen from a large national study. The *Campylobacter* contamination on the skin of whole chicken at retail was monitored over 12 months in the UK (Jorgensen et al. 2015). This study examined *Campylobacter* concentrations on 4011 chickens across all retail outlets between February, 2014 and March, 2015. Analysis of the raw data[2] gave a mean log-concentration of 1.83 $\log_{10}$ cfu/g (Sd: 1.36 $\log_{10}$ cfu/g). This demonstrates that variability in the concentration of *Campylobacter* on chicken at retail level is large with 5.5% of samples ≥4.0 $\log_{10}$ cfu/g.

19.2.3.4 Home Cooking

Exposure to *Campylobacter* from chicken meat prepared in a domestic kitchen depends on the ability of cooking to inactivate the microorganism as well as the stringency of hygienic handling and preparation practices to prevent cross contamination of cooked chicken meat or other ready-to-eat foods. The heat resistance of *Campylobacter* is not generally considered to be high. Several studies have published D-values that describe the heat inactivation of *Campylobacter*. These were used to calculate a general D-value for the microorganism (Van Asselt and Zwietering 2006). They calculated a D_{70}-value of 0.11 min (6.58 s) with a 95% confidence interval of 1.12 min and a z-value of 12.3 °C.

Since then, two studies have suggested much higher heat resistance for *Campylobacter* on chicken fillets when cooked (De Jong et al. 2012; Bergsma et al. 2007). Chill storage temperatures and the chicken meat itself along with fast heating rate could contribute to the enhanced heat resistance (De Jong et al. 2012). Such findings suggest that cooking may affect the incidence of illness due to *Campylobacter* on chicken more than is usually considered in risk assessments.

[2] "Counts recorded as <10 cfu/g were set at 1 cfu/g before $\log_{10}$ transformation and fitting to a normal distribution using @Risk™"

However, cooking practices are variable in the home and undercooking is also a distinct possibility. For example, a study in U.S. monitored home cooking temperatures for poultry (Audits International 2000) and found the mean internal temperature to which consumers cooked their poultry was 158.6 °F (70.3 °C) with a standard deviation of 20.58 °F (11.43 °C). Hence, there was considerable variability in the internal temperature achieved when consumers considered that their poultry was cooked (95% CI 47.47–93.19 °C).

It should also be clear that the *Campylobacter* contaminating chicken meat are not homogeneously distributed throughout the whole muscle meat. Rather, the majority are situated on the outside where they would experience elevated temperatures for longer times. In a study of 100 skinless, deboned chicken fillets, Luber and Bartelt (2007) found that prevalence of *Campylobacter* on the surface was 87% in contrast to 20% in the deep tissue. They reported that the mean number of *Campylobacter* on the surface of the fillets was 1903 cfu, while the mean *Campylobacter* counts inside the tissue was 0.24 cfu; over 4000 times lower. Clearly this would increase the amount of *Campylobacter* killed by any specific cooking treatment.

19.2.3.5 Cross Contamination During Home Preparation

Campylobacter from chicken meat are likely to be transferred to hands and surfaces during handling and preparation in the domestic kitchen. Recommended interventions such as washing hands and cleaning and disinfection of surfaces and equipment like knives, if adhered to, will eliminate or greatly reduce this problem. However, adherence to these recommendations is not universal and there have been studies that have attempted to quantify the consequences of non-adherence. A good example of a cross-contamination study was carried out in Germany to examine the quantity of *Campylobacter* that was transferred during the preparation of raw chicken meat (Luber et al. 2006). One scenario quantified the percent transfer of *Campylobacter* from the surface of five chicken breast fillets during slicing to hands, the chopping board, and the blade of the knife and to cucumber slices cut with the same equipment without cleaning. Luber et al. (2006) found that on average 3.8% of *Campylobacter* transferred from the fillets to the hands (SD = 5.9%), 1.1% transferred from the fillets to the board and knife (SD = 0.7%) and 10.3% of *Campylobacter* on the board and knife transferred to the sliced cucumber when the same equipment was used (SD = 9.6%). Hence the proportion of *Campylobacter* transferred from raw chicken to cucumber is 0.001133 (SD = 0.096). Most quantitative risk assessments conducted for *Campylobacter* on chicken have attempted to model cross-contamination because it is considered a major route of exposure.

19.2.4 Risk Characterization

Campylobacteriosis resulting from the consumption of poultry has been the subject of several quantitative microbial risk assessments (QMRA). These have variously targeted the whole or specific parts of the poultry production chain, and have been conducted on national and international levels.

The first full food chain QMRA for *Campylobacter* in poultry was conducted in Canada (Fazil et al. 1999) and this was followed by a U.K. farm to fork model (Hartnett et al. 2001) and QMRAs published in The Netherlands between 2005 and 2007 (Nauta 2005; Havelaar et al. 2007; Nauta 2007). Other QMRAs focusing on a slaughter to consumption approach were created for Denmark (Rosenquist et al. 2003) and New Zealand (Lake et al. 2007). The first international farm to fork QRA on the subject was published by FAO/WHO in 2009 (FAO/WHO 2009), and this was followed in 2011 by a Europe-wide farm to fork QMRA conducted by the European Food Safety Authority (EFSA 2011). Other short chain QMRAs of note are the retail to consumption approach applied in Belgium

(Uyttendaele et al. 2006) and the post-chill production to consumption model in Sweden (Lindqvist and Linblad 2008). These both have a particular focus on consumer handling and preparation of chicken which is a major risk factor in campylobacteriosis. A comparison of the European full chain QMRAs for *Campylobacter* in chicken meat before 2009 describes their technical bases and their conclusions (Nauta et al. 2009).

19.3 Risk Management

The data used in this section have been selected from the literature cited previously in this chapter to provide an illustration of the establishment of appropriate risk management metrics to facilitate the control of campylobacteriosis resulting from the consumption of chicken meat. Other values could be selected with different outcomes. Hence the ALOP, FSOs and POs calculated in the following sections should not be applied in practice without further consideration.

19.3.1 Acceptable Level of Consumer Protection

One of the most comprehensive studies of foodborne disease statistics was conducted in the U.S. Scallan et al. (2011) reported that there were 43,696 confirmed cases of campylobacteriosis in the U.S. and estimated that 845,024 domestically acquired illnesses resulted from the consumption of food in the U.S. after accounting for under-reporting and under-diagnosis (90% credible interval 337,031–1,611,083).

However, chicken meat is only one source of campylobacteriosis in the population and to set risk management metrics for chicken production, it is necessary to define an ALOP that relates only to campylobacteriosis from the consumption of chicken meat (Zwietering 2005) rather than other sources. In the European Union it has been estimated that between 20% and 30% of total confirmed human campylobacteriosis cases are associated with the handling, preparation and consumption of chicken meat (EFSA 2010a).

Assuming the same attribution proportion is also applicable to the situation in the U.S. this suggests that there were 848 foodborne cases of campylobacteriosis per million population[3] attributable to the handling, preparation and cooking of chicken. This value will be used as the ALOP for the purposes of the illustrated example.

19.3.2 Establishing a Food Safety Objective

Zwietering (2005) described a scheme for the calculation of a food safety objective (FSO) from an ALOP. The maximum number of cases per unit population (ALOP) is related to the number of servings of chicken consumed per person per year (S), the probability of illness following ingestion of one *Campylobacter* cell (r) and the dose (number of *Campylobacter* cells) consumed on an eating occasion (D) (see Eq. 19.2).

$$\mathrm{ALOP} = \mathrm{S}^{*}\,1\mathrm{E}6^{*}\,\mathrm{r}^{*}\,\mathrm{D} \tag{19.2}$$

[3] Based on a population 299 million for the U.S. (Scallan et al. 2011)

The dose (D) is directly related to the mass of chicken meat consumed (M) on an eating occasion and the concentration of *Campylobacter* cells per gram of chicken meat at the point of consumption (FSO) in colony forming units per gram (cfu/g). Hence, Eq. 19.2 can be rearranged to calculate the FSO (Eq. 19.3).

$$\mathrm{FSO} = \log_{10}\left(\mathrm{ALOP} / \left(\mathrm{S}^{*}\,1\mathrm{E}6^{*}\,\mathrm{r}^{*}\,\mathrm{M}\right)\right) \tag{19.3}$$

The FAO/WHO risk assessment for *Salmonella* on chicken meat provides detailed data on chicken meat consumption patterns (FAO/WHO 2002). It specified, based on Irish food consumption data, that chicken meat was eaten on average, 2.04 times per week which equates to 106.08 servings (S) per year. In the same report the median weight of cooked chicken meat consumed was approximately 100 g (M).

The probability of infection (P_{inf}) given a dose of 1 *Campylobacter* cell (N) can be calculated from the Beta-Poisson dose response model in Eq. 19.4.

$$P_{\mathrm{inf}} = 1 - \left(1 + \mathrm{N} / \beta\right)^{-\alpha} \tag{19.4}$$

The value of beta and alpha which are the dose response parameters for *Campylobacter*, are 59.95 and 0.21 respectively (FAO/WHO 2009). Substituting these values into Eq. 19.4, results in a P_{inf} value of 0.003468 or a 1 in 288 chance of being infected having consumed a single *Campylobacter* cell.

The probability of illness given infection is somewhat lower than this as not all people infected with *Campylobacter* will develop illness due to immunity and other host defences. From the feeding trials published by Black et al. (1988) as reproduced by FAO/WHO (2009) the average probability of illness (P_{ill}) given infection can be calculated as 0.325843 based on the total number of people ill given the total number of people infected across all trials. So approximately 1 person will get ill for every 3 infected through consumption of a single *Campylobacter* cell. The probability of illness given infection (r) is therefore a product of P_{ill} and P_{inf} which is 0.00113, meaning that, on average, 1 person in 885 people who consume a single *Campylobacter* cell will develop campylobacteriosis.

Substituting the values for the number of servings per annum (S), the mass of a serving in grams (M), the probability of illness given ingestion of a single *Campylobacter* cell (r) and the target ALOP (848 cases per million population (see Sect. 19.3.1)) into Eq. 19.3 results in an FSO of - 4.15 $\log_{10}$ cfu/g (or 7.074E-5 cfu/g) cooked chicken meat as consumed.

19.3.3 Quantifying a Public Health Goal

Public health authorities around the world, tasked with the improvement of public health, seek to reduce the number of foodborne illnesses suffered by their respective populations. In this regard they often express a public health goal in qualitative terms e.g. reducing foodborne illness. However, the risk management metrics adopted internationally (Codex Alimentarius Commission 2007), allow for a risk management approach that can meet a quantitative public health goal. For the purposes of illustration the public health goal that will be adopted here is a 50% reduction in the ALOP (see Sect. 19.3.1). Hence, the maximum number of cases of campylobacteriosis per million population needs to be reduced to 424. Following the steps set out in Sect. 19.3.2 and replacing the ALOP with the new public health goal leads to a requirement for the poultry production industry to meet an FSO of - 4.45 $\log_{10}$ cfu/g (3.537E-5 cfu/g) cooked chicken meat as consumed.

19.3.4 Control Measures to Achieve a Public Health Goal

The control of *Campylobacter* starts at primary production where the focus is on the prevention of horizontal spread. Many control measures have been suggested as referred to in Sect. 19.2.3. However, it is clear that the practice of thinning breaches biosecurity and increases the prevalence of *Campylobacter* on poultry at the point of slaughter.

At slaughter, three key operations emerge as potential control points for the reduction of *Campylobacter* on chicken carcasses. The scalding step reduces the number of organisms on the chickens prior to evisceration and the temperature of the tanks dictates the size of the reduction. The washing step can result in further reductions that are enhanced if antimicrobials are used. Finally, the chilling step, if carried out by water emersion with antimicrobials in the chilling water, will also reduce the number of *Campylobacter* on the chilled carcasses.

During retail distribution and storage, freezing can help to reduce *Campylobacter* numbers on carcases although chill distribution temperatures and times in air seem to result in no appreciable reduction.

A very important control measure with regard to *Campylobacter* in fresh chicken meat is thorough cooking by the consumer. Unfortunately this control measure is subject to a high level of variability and cross contamination resulting from the handling and preparation of the raw meat in the domestic kitchen can undermine the control exerted by the cooking step, either by transfer of *Campylobacter* onto the cooked meat or by transfer of *Campylobacter* onto other ready-to-eat foods.

19.3.5 Establishing Performance Objectives Throughout the Chicken Production Chain to Account for Control Measures

Performance objectives (POs) are targets at specific points in the food chain by which food businesses can ensure that their processing control measures are sufficient to meet the FSO. They are flexible in that the food business can employ any number and combination of control measures as long as the PO is achieved. Hence POs can contribute to improvements in public health while allowing innovation in process control.

The following sections show examples of how risk managers can set POs for different points in the chicken production chain to meet the public health goal FSO of -4.45 $\log_{10}$ cfu/g (see Sect. 19.3.2).

19.3.5.1 Influencing Consumer Handling and Cooking Practices in the Home

Food businesses can influence and sometimes control consumer handling and cooking practices so that the public health goal FSO can be met. This involves influencing cooking practice, influencing or controlling the probability of cross-contamination during handling of chicken and also by controlling the concentration of *Campylobacter* on raw chicken at the end of the retail distribution chain before the consumer buys it.

The two most significant consumer preparation practices that affect *Campylobacter* levels on chicken at the point of consumption are cooking and handling. The latter practice is a common source of potential cross-contamination. The scale of log-reduction ($\sum$R) will be exclusively driven by consumer cooking and the scale of log-increase ($\sum$I) will be driven by cross-contamination because *Campylobacter* will not grow at ambient temperatures and therefore storage temperature abuse in the home is unlikely to have any impact on $\sum$I. This illustrated example will be based on the information provided in Sect. 3.5.2.

Normally, in the application of the ICMSF equation, the log-increase in numbers ($\sum$I) and the log-decrease in numbers of *Campylobacter* ($\sum$R) can be summed and the change in the starting log-concentration (H_0) can be calculated. However, when considering cross-contamination of cooked chicken from the kitchen surfaces, utensils and hands after cooking, the home preparation step has to be broken into two sub-steps and the calculations carried out in sequence; first the effect on log –concentration of cooking and then the effect of cross- contamination after cooking on the log-concentration of *Campylobacter* on the cooked meat.

Effect of Cooking

The U.S. FDA recommends cooking poultry to an internal temperature of 165 °F (79.3 °C). This was based on an expert elicitation by the US National Advisory Committee on Microbiological Criteria for Foods (NACMCF 2007). That document refers to the FSIS time temperature tables for reduction of *Salmonella* where165°F (73.9 °C) for 10 s is cited as achieving a 7-log-reduction. *Campylobacter* is considered to be more heat susceptible than *Salmonella*. (Van Asselt and Zwietering 2006) and therefore it is reasonable to assume that the U.S. consumer cooking recommendation would result in >7-log reduction in *Campylobacter*. Hence, for the purposes of illustration it is reasonable to assume that on average at least a 7 $\log_{10}$ cfu/g reduction ($\sum$R) in *Campylobacter* would be achieved throughout the meat. Because there is no growth in *Campylobacter* prior to cooking in the consumer's home, $\sum$I is negligible and can be ignored. In the absence of cross-contamination, the maximum concentration of *Campylobacter* that can be tolerated on the raw chicken as it enters the consumers home, $H_{0\text{-}c}$ (the hazard level at the start of consumer step), that would still facilitate achievement of the public health goal FSO, can be calculated using Eq. 19.5.

$$H_{0-c} \leq FSO + \sum R \leq -4.45 + 7 \leq 2.55 \log_{10} \text{cfu/g} \tag{19.5}$$

Therefore, if cross contamination in the home is negligible a PO for the level of *Campylobacter* on raw chicken before it enters the consumer's home would be $\leq H_{0\text{-}c}$ which in this example is 2.55 $\log_{10}$ cfu/g. This level would be sufficient to meet the public health goal FSO of −4.45 $\log_{10}$ cfu/g with a cooking step delivering at least a 7 $\log_{10}$ reduction.

As a control measure food businesses can contribute to correct cooking in the home by including clear cooking instructions on the packaging of the chicken that do not require packaging to be removed in order to read them (e.g. instructions on the outside of labels).

The Effect of Cross-Contamination

Unfortunately, cross contamination in the home during preparation of chicken happens all too frequently in consumers' kitchens. For the purposes of this illustration, if it is assumed the consumer's kitchen is clean before handling the chicken, the scale of cross-contamination is dependent on the log concentration of *Campylobacter* on the raw chicken prior to preparation for cooking ($H_{0\text{-}c}$) and the amount transferred back onto the cooked chicken (and other foods) by the consumer during poor handling practices.

Zwietering (2005) discussed the incorporation of a term for cross contamination into the ICMSF equation. To apply the ICMSF equation to cross contamination the log-increase $\sum$I must be subdivided into the sum of growth ($\sum$G) and the sum of cross-contamination ($\sum$C). However, unlike $\sum$G and $\sum$R, $\sum$C is dependent on the starting concentration and is 'additive' with respect to the ICMSF equation, on the linear scale rather than the logarithmic scale. (see Chap. 3, Sect. 3.5.2).

Zwietering (2005) proposed a way of calculating the addition of bacteria to food due to cross contamination. However, in considering cross-contamination in the home it is also important to consider that it does not always take place and hence the prevalence of a cross-contamination event is less than 100% (proportion < 1). This means that not all servings of chicken carry *Campylobacter* as a result of cross contamination. To account for this, the concentration transferred during cross contamination needs to be multiplied by the proportion of cross contamination events. Eq. 19.6 is the resulting modification of the equation proposed by Zwietering (2005).

$$C = \log_{10}\left(\left(\left(10\,\hat{}\,\left(H_{0-c} - \Sigma R\right)\right) + \left(\left(10\,\hat{}\,H_{0-c}\right)^{*}\,Tr^{*}\,p\right)\right) / \left(10\,\hat{}\,\left(H_{0-c} - \Sigma R\right)\right)\right) \tag{19.6}$$

Where: C = log increase in concentration due to cross contamination ($\log_{10}$ cfu/g); $H_{0\text{-}c}$ = Starting log concentration of *Campylobacter* on the raw chicken prior to preparation for cooking ($\log_{10}$ cfu/g); $\sum$R = log reduction due to cooking; Tr = transfer rate of *Campylobacter* added to the food by the cross contamination event (value between 0 and 1); *p* = proportion of cross contamination events happening in the population of all preparation events (value between 0 and 1).

To calculate the net effect of the cooking and cross contamination step it is necessary to modify the general ICMSF equation as shown in Eq. 19.7.

$$H_{0-c} - \sum R + \sum C \leq FSO \tag{19.7}$$

In the example illustrated in this section where cooked chicken is cross contaminated after cooking by bacteria originating on the raw chicken, $\sum$R is a 7 $\log_{10}$ reduction due to cooking, and Tr is the extent of *Campylobacter* contamination transferred from the raw chicken to the cooked chicken via the chopping board and knife using the transfer rate published by Luber et al. (2006) (see Sect. 19.2.3.5). Table 19.1 shows the consequences of different starting log concentrations of *Campylobacter* on raw chicken ($H_{0\text{-}c}$) and the proportion of cross contamination events (*p*) on attaining the desired public health goal FSO (−4.45 $\log_{10}$ cfu/g) using Eqs. 19.6 and 19.7.

If the consumer handling and cooking pathway is considered as a whole then it is clear that thorough cooking, in the absence of poor handling practices, could facilitate the tolerance of higher starting concentrations of *Campylobacter* on raw chicken entering the consumer's home (e.g., up to 2.55 $\log_{10}$ cfu/g) but if the likelihood of cross-contamination is anything other than very low (see Table 19.1) then the public health goal FSO will not be met by this value and more cases of campylobacteriosis observed in the population than tolerable can be expected.

Table 19.1 Calculation of FSO for different cross contamination scenarios[a, b]

Fixed values				
∑R (cooking)	7.00		Tr	0.001133

	Cross contamination prevalence (*p*)				
$H_{0\text{-}c}$ ($\log_{10}$ cfu/g)	**1**	**0.1**	**0.01**	**0.001**	**0.0001**
3.00	0.05	-0.95	-1.95	-2.95	-3.95
2.00	-0.95	-1.95	-2.95	-3.95	-4.95
1.00	-1.95	-2.95	-3.95	-4.95	-5.95
0.50	-2.45	-3.45	-4.45	-5.45	-6.45
0.10	-2.85	-3.85	-4.85	-5.85	-6.85

[a]See Sect. 19.2.3.5, [b]FSO calculated using Eqs. 19.6 and 19.7. Shaded area shows values for *p* and $H_{0\text{-}c}$ that meet the public health goal FSO of −4.45 log10 cfu/g

Studies show that cross contamination in the home is frequent. For example a study of domestic food preparation involving the handling of raw chicken demonstrated that >50% of subjects failed to properly decontaminate chopping boards and utensils between their use in preparing raw chicken and subsequent use in the preparation of other ready-to-eat food (Redmond et al. 2004). Food businesses can contribute to improved handling of raw chicken by consumers by including clear safe handling instructions on the packaging of raw chicken. This should include a warning not to wash chicken as this is a known source of *Campylobacter* spread throughout the kitchen. Authorities can also contribute to improved consumer behaviour with regards to the handling and cooking of chicken in the home through national education campaigns. However, it is unlikely that education and labelling alone could achieve the low rate of cross-contamination necessary to deal with a high concentration of *Campylobacter* on the raw chicken.

One way to achieve a very low cross-contamination rate in the home is to ensure consumers do not come into contact with raw chicken. Food businesses can achieve this by packing chicken in heat resistant packaging where the chicken can be cooked in pack without handling. The technology to do this for whole chicken carcasses exists, and is being applied by some manufacturers in Europe. This control measure would result in an extremely low prevalence rate of cross-contamination and therefore, the public health goal FSO would be met by the ability of the cooking pathway to reduce the level of *Campylobacter* on the raw chicken, assuming that recommended cooking instructions are followed. Using this packaging technology, the PO at the end of retail distribution (PO_r) could be as high as 2.55 $\log_{10}$ cfu/g and the public health goal FSO would still be met.

Where packaging solutions to prevent cross-contamination are not feasible or not acceptable to all consumers, then it may be necessary to set a more stringent PO at the end of retail for *Campylobacter* on raw chicken by controlling the level on raw chicken achieved by processing steps earlier in the chain. The $H_{0\text{-}c}$ values shown in Table 19.1 could be considered as potential PO_r values at the end of retail distribution. If cross contamination in the home is assumed to be very low at only 0.1% or 1 in 1000 chicken preparation events, then calculation $H_{0\text{-}c}$ using equations 6 and 7 indicates that an appropriate PO_r for raw chicken at the end of retail distribution would be 1.495 $\log_{10}$ cfu/g.

Conformance of chicken batches with the PO_r can be verified using a suitable microbiological criterion. However, testing for *Campylobacter* on chicken is usually done by quantifying the numbers on the surface of the carcass. This is either done by a whole carcass rinse technique (normally 100 ml rinse volume) or by taking a 25 g mass of chicken neck skin for microbiological testing. The PO value refers to the concentration per gram of chicken rather than per millilitre carcass rinse or gram of skin. Therefore to operationalize the PO it is necessary to convert the PO concentration to a concentration per millilitre carcass rinse or gram of skin.

A 1.5 kg whole chicken has approximately 988 g of meat and skin (818 g meat and 85 g skin) (ACMF 2013). Hence if all of the *Campylobacter* are assumed to be distributed on the skin then a chicken meeting the PO_r of 1.495 $\log_{10}$ cfu/g edible chicken would have less than or equal to a PO_r of 2.49 $\log_{10}$ cfu/ml carcass rinsate (assuming 100 ml rinse used) or 2.56 $\log_{10}$ cfu/g skin.[4] However, in a batch of chickens there will be a distribution of *Campylobacter* numbers (in $\log_{10}$) on the carcass and therefore it is necessary to limit the number of carcasses that exceed the PO_r to a small proportion of the batch in order to meet the FSO at the point of consumption. Therefore, a risk management decision has to be made regarding the proportion of chickens that may exceed the PO_r. For illustration this value will be set at 1%. Therefore, a batch of chickens that would only exceed the PO_r 1% of the time would have to have a batch mean that was 2.33 times the standard deviation for the batch lower than the PO_r. If the standard deviation for the batch is assumed to be 0.6 $\log_{10}$ cfu/ml carcass rinsate, the batch mean would have to be 1.092 $\log_{10}$ cfu/ml carcass rinsate (see Fig. 19.1).

[4] *Campylobacter* level = 1.495 $\log_{10}$ cfu/g chicken, therefore 988 g of meat and skin would contain 30,886 *Campylobacter* cfu. If all on skin then $\log_{10}(30{,}886/85)$ = 2.5603 $\log_{10}$ cfu/g skin or $\log_{10}(30{,}886/100)$ = 2.4897 $\log_{10}$ cfu/ml carcass rinse water.

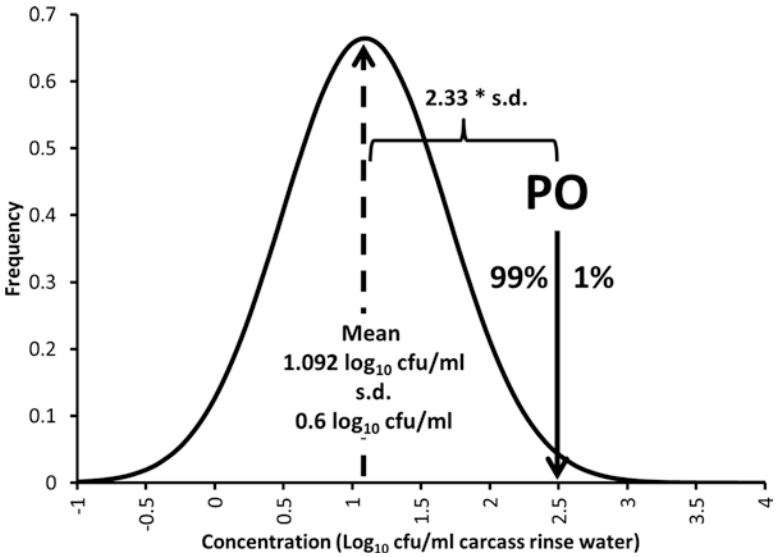


Fig. 19.1 Distribution of *Campylobacter* (log cfu/ml) in the carcass rinsate for a batch of raw chickens that meets the PO_r at retail

The ICMSF spreadsheet tool (www.ICMSF.org) can be used to calculate an MC to verify the distribution of log-concentrations shown in Fig. 19.1 with 95% confidence. An example of such an MC could be a 3-class plan where n = 7 c = 1 m = 1 $\log_{10}$ cfu/ml M = 2 $\log_{10}$ cfu/ml. Clearly the lower the standard deviation of the batch the closer the mean log-concentration of the batch can be to the PO_r. Hence it is important to reduce the variability in the batch log-concentrations as much as possible by consistent controlled processing.

19.3.5.2 Control Measures During Retail Distribution

Control measures during retail distribution can impact on the concentration of *Campylobacter* on raw chicken at the end of the retail distribution chain. This is achieved by controlling the temperature of the distribution chain in combination with controlling the concentration of *Campylobacter* on chicken entering the retail distribution chain from the slaughter plant. Section 19.2.3.3 discussed the fate of *Campylobacter* during retail storage and distribution.

The effect of the retail chill distribution chain on the survival of *Campylobacter* is varied and results differ between studies using inoculated chicken and those using natural contamination. For example, Georgsson et al. (2006) found no significant reduction in naturally contaminated chicken carcasses stored at 3 °C for 7 days. Therefore chill distribution of chicken at retail cannot be considered to be a reliable control measure for *Campylobacter* and the only control measure currently available to the authorities or the retailer, is to set a stringent PO for raw chicken leaving the slaughter plant (PO_s) so that concentrations can be controlled to meet the PO_r set at the end of the retail distribution chain. Here, an appropriate PO_s at the end of slaughter for chicken destined for chill distribution at retail should be the same at the PO_r at the end of retail, namely $PO_s \leq 2.49$ $\log_{10}$ cfu/ml carcass rinsate (see Sect. 19.3.5.1).

In contrast, frozen retail storage and distribution chains result in a decrease in *Campylobacter* numbers from the end of slaughter to the consumer's home. Reports on the effect of freezing on *Campylobacter* vary in the magnitude of the effect and also seem to be influenced by whether natural

contamination or inoculated laboratory strains were the source of *Campylobacter* on the chicken. The study by Georgsson et al. (2006) provides a reasonable basis for quantification of the effect of freezing on *Campylobacter*. They reported that frozen storage over 31 days at -20 °C achieved a log-reduction in *Campylobacter* on chickens from 5 flocks, of 0.65 to 2.87 with an average log-reduction across the five flocks, of 1.77 (sd = 0.79).

This intervention allows food businesses to accept a higher concentration of *Campylobacter* on raw chicken entering the retail frozen distribution chain from the slaughter plant (PO_s at the end of slaughter is the $H_{0\text{-fd}}$ at the beginning of the frozen distribution chain) while allowing the product to meet the PO_r at the end of retail distribution. The acceptable PO_s can be calculated using the ICMSF equation (Eq. 19.8).

$$PO_s = H_{0-fd} \leq PO_r - \sum R \leq 2.49 + 1.77 \leq 4.26 \log_{10} \text{cfu / ml} \tag{19.8}$$

Where $PO_s = H_{0\text{-fd}}$ = level (log cfu/ml) of *Campylobacter* on chicken at the end of slaughter destined for frozen distribution at retail; PO_r = Performance Objective at the end of retail and $\sum R$ = the sum of log reductions due to frozen distribution (mean data taken from Georgsson et al. (2006)).

Therefore, if frozen distribution at retail is chosen as an intervention to allow the raw chicken to meet the PO_r at the end of retail distribution, then a PO_s can be established for raw chicken leaving the slaughter plant which is equivalent to the $H_{0\text{-fd}}$ calculated in Eq. 19.8. That is, $PO_s = 4.26$ $\log_{10}$ cfu/ml carcass rinsate.

As discussed previously, a risk management decision has to be made regarding the proportion of chickens that may exceed the PO_s at the end of slaughter for frozen retail distribution. For illustration this value will again be set at 1%. Therefore, a batch of chickens that would only exceed the PO_s 1% of the time would have to have a batch mean that was 2.33 times the standard deviation for the batch lower than the PO_s. If the standard deviation for the batch is assumed to be 0.6 $\log_{10}$ cfu/ml carcass rinsate the batch mean would have to be 2.86 $\log_{10}$ cfu/ml carcass rinsate (see Fig. 19.2).

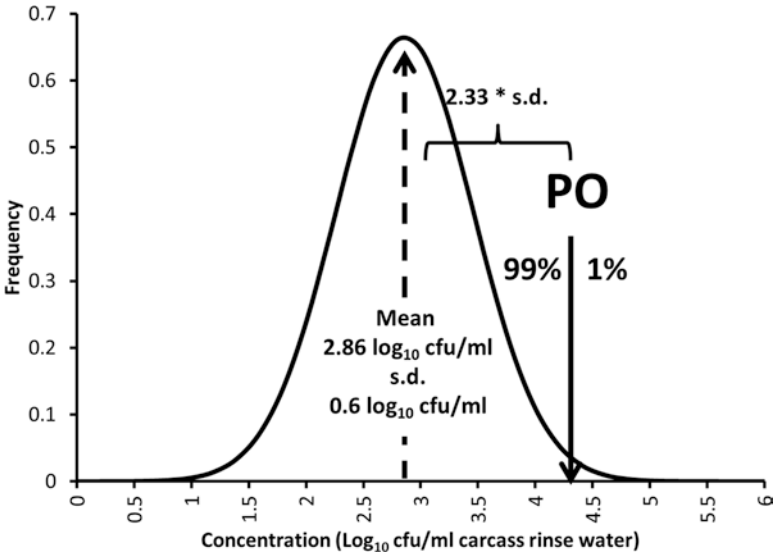


Fig. 19.2 Distribution of *Campylobacter* in the carcass rinsate (Log_{10} cfu/ml) for a batch of raw chickens that meets the PO_s at the end of slaughter for chicken destined for frozen retail storage and distribution

The ICMSF spreadsheet tool (www.ICMSF.org) can be used to calculate an MC to verify the distribution of log-concentrations shown in Fig. 19.2 with 95% confidence. An example of such an MC could be a 3-class plan where n = 10 c = 1 m = 3 $\log_{10}$ cfu/ml M = 4 $\log_{10}$ cfu/ml. Clearly the lower the standard deviation of the batch the closer the mean log-concentration of the batch can be to the PO_s. Hence it is important to reduce the variability in the batch log-concentrations as much as possible by controlled processing.

In countries where there is consumer demand for both chill and frozen chicken a possible risk management approach by the retailer in collaboration with the slaughter plant, could be to test batches of chicken at the end of slaughter using the MCs proposed here and if they cannot meet the PO_s for chill distribution then they are frozen and diverted to frozen distribution provided they meet the PO_s for frozen distribution. However, this would be dependent on the feasibility of holding carcasses pending results from microbiological testing.

19.3.5.3 Control Measures During Slaughter and Processing

Section 19.2.3.2 discussed studies that have investigated the effect of different slaughter and processing interventions on the concentration of *Campylobacter* on chicken. Combinations of these can be used in conjunction with controls on the level of *Campylobacter* on the birds received from the farm before the scalding step ($H_{0\text{-}f}$). Table 19.2 summarizes, in ICMSF notation, the sum of log-increases ($\sum$I) and the sum of log-reductions ($\sum$R) in *Campylobacter* ($\log_{10}$ cfu/g) based on the minimum mean change reported for given interventions in the studies cited.

In some jurisdictions there are legal restrictions on the use of some of these processes. For example, in Europe legislation limits the use of antimicrobials during slaughter and processing. Therefore antimicrobial sprays cannot be used. Emersion chilling is also not a viable option under such circumstances when antimicrobials like chlorine cannot be used to prevent cross-contamination amongst chicken carcasses from the chill water. Nevertheless, processors have to ensure that their process is running hygienically and that the equipment is set correctly to ensure the best microbiological outcome for the finished product. For example, if plucking and evisceration machinery is not set correctly for the size of carcass being handled then increases in *Campylobacter* concentrations on chicken carcasses are inevitable. These increases are clearly shown in a number of studies reviewed by Guerin et al. (2010).

Clearly there is a range of possible levels of *Campylobacter* on chickens prior to slaughter ($H_{0\text{-}f}$) that could be tolerated to achieve the desired PO_s at the end of slaughter. These are summarised in Table 19.3 along with suitable microbiological criteria (assuming sd. 0.6 $\log_{10}$ cfu/ml carcass rinse and a 95% confidence limit that only 1% of the batch would exceed the PO_s) based on a series of processing steps taken from Table 19.2.

Table 19.2 Poultry slaughter steps and their effect on mean *Campylobacter* concentrations on chicken carcasses (minimum reported values)

Processing step	$\sum$I ($\log_{10}$ cfu)	$\sum$R ($\log_{10}$ cfu)	Reference
1. Scalding	No significant change	1.54 (sd. 0.29)	Duffy et al. (2014)
2. Defeathering/ evisceration	No significant change	No change	Duffy et al. (2014)
3. Carcass washing	No significant change	0.26 (sd. Not reported)	Bashor et al. (2004)
4a. Antimicrobial treatment TSP	No significant change	1.03 (sd. Not reported)	Bashor et al. (2004)
4b Antimicrobial treatment ASC	No significant change	1.26 (sd. Not reported)	Bashor et al. (2004)
5a Emersion chilling	No significant change	1.02 (sd. 0.3)	Duffy et al. (2014)
5b Air chilling	No significant change	0.2 (sd. Not reported)	Guerin et al. (2010)

See Sect. 19.2.3.2 for full details of treatments and range of concentration changes reported

Table 19.3 Range of possible *Campylobacter* levels (log_{10} cfu/g or ml) for birds prior to scalding (H_{0-f}) for chicken carcasses before slaughter based on slaughter steps employed and retail distribution chain selected

Process step sequence[a]	$\sum R$ (log_{10} cfu)	H_{0-f} to meet the PO_s for retail chill[b] (log_{10} cfu/ml)	MC[c] to meet PO_s for retail chill[b]	H_{0-f} before slaughter to meet the PO_s for retail frozen[d] (log_{10} cfu/ml)	MC[c] to meet PO_s for retail frozen[d]
1,2,3,5a	2.82	5.31	n = 6 c = 1 m = 3.5 M = 4.5	7.01	n = 5 c = 2 m = 5.0 M = 6.5
1,2,3,4a,5a	3.85	6.34	n = 5 c = 2 m = 4.5 M = 5.5	8.11	n = 5 c = 1 m = 6.5 M = 7.5
1,2,3,4b,5a	4.08	6.57	n = 6 c = 1 m = 5.0 M = 6.0	8.34	n = 5 c = 2 m = 6.5 M = 7.5
1,2,3,5b	2.00	4.49	n = 5 c = 2 m = 2.5 M = 4.0	6.26	n = 6 c = 2 m = 4.5 M = 6.0
1,2,3,4a,5b	3.03	5.52	n = 5 c = 2 m = 3.5 M = 4.5	7.29	n = 6 c = 2 m = 5.5 M = 6.5
1,2,3,4b,5b	3.26	5.75	n = 6 c = 2 m = 4.0 M = 5.0	7.52	n = 5 c = 2 m = 5.5 M = 7.0

[a]See Table 19.2 for process numbers
[b]PO_s after slaughter for chicken destined for chill distribution at retail (2.49 log_{10} cfu/ml)
[c]Microbiological criterion where m and M are in log_{10} cfu/ml carcass rinse
[d]PO_s after slaughter for chicken destined for frozen distribution at retail (4.26 log_{10} cfu/ml)

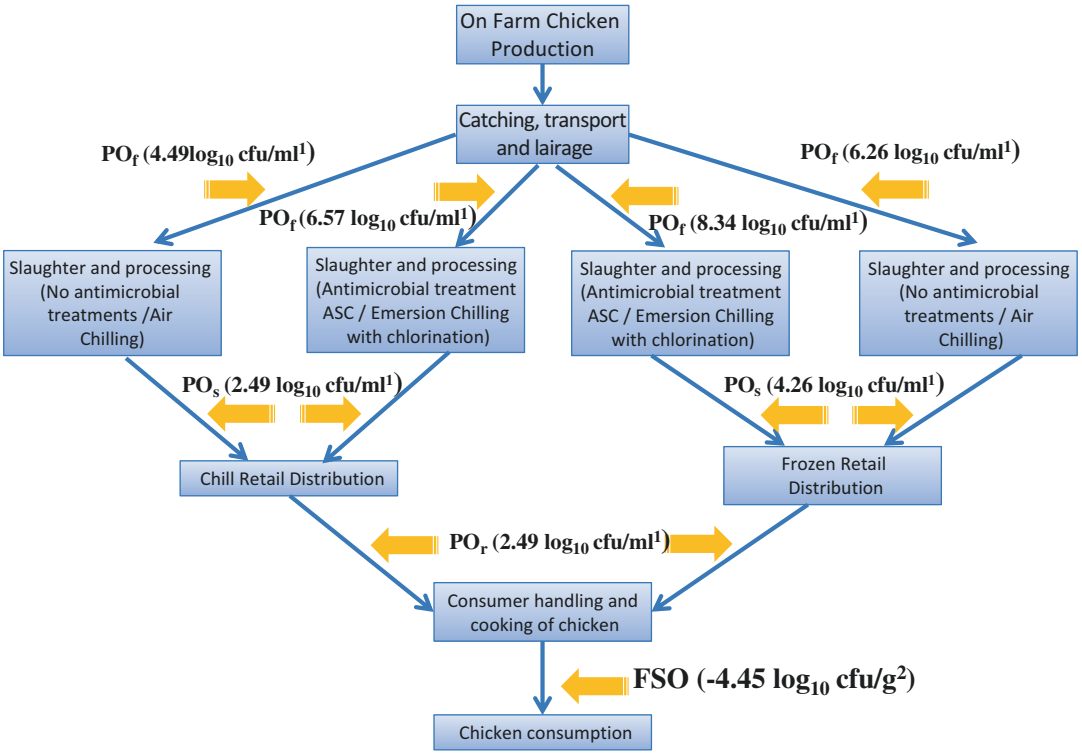
Table 19.3 shows that a higher H_{0-f} for the chicken prior to scalding can be tolerated as long as there is a multi-intervention approach during processing. For example, a combination of scalding, defeathering, evisceration, carcass washing, antimicrobial treatment with ASC spray and emersion chilling can tolerate batches of birds with *Campylobacter* concentrations of 6.58 log $_{10}$ cfu/ml carcass rinsate. Whereas, if the antimicrobial treatment step is omitted and emersion chilling is replaced with air chilling then the H_{0-f} prior to slaughter would have to be approximately two log_{10} units lower. This would mean that on farm biosecurity measures and hygiene control of farm to slaughter transport and lairage would have to be much more stringent to achieve the PO_s at the end of slaughter for chicken destined for the retail chill distribution chain. Alternatively, such chicken might have to enter the retail frozen distribution chain where the PO_s at the end of slaughter is higher (less stringent). On farm biosecurity measures are discussed in more detail in Sect. 19.2.3.1. However, if thinning is practiced it is unlikely that the lower H_{0-f} values at slaughter could be achieved.

The authorities or processors could set a PO_f at the start of slaughter that would be equivalent to the H_{0-f} tolerable for the slaughter and processing steps employed as well as the retail distribution method required by the market. For example, a typical European slaughter and processing step that serves the retail chill distribution chain would have to target a PO_f of 4.50 log_{10} cfu/ml carcass rinsate before scalding.

A difficulty with operationalizing a PO_f prior to slaughter is caused by the normal method of microbial testing of chicken batches before slaughter. This involves cecal samples from the flock and there is no means of converting these *Campylobacter* concentrations into concentrations per millilitre carcass rinse water. Some means of relating cecal counts to the PO_f would have to be found or else carcass rinse testing methods would have to be employed before the scalding step.

19.3.5.4 Summary of Performance Objective Approach to the Control of Campylobacter in Chicken

Setting performance objectives at specific points in the chicken production chain allows food businesses the flexibility to establish their processes in the way most suited for them to ensure that the FSO is met. Sections 19.3.5.1, 19.3.5.2 and 19.3.5.3 have used an illustrative approach to demonstrate how such a performance objective led control process could be developed. The illustration is summarized in Fig. 19.3 and shows clearly where decisions have to be made regarding the intervention steps employed in the chicken production chain.


On Farm Chicken Production
Catching, transport and lairage
PO$_f$ (4.49log$_{10}$ cfu/ml[1])
PO$_f$ (6.57 log$_{10}$ cfu/ml[1])
PO$_f$ (8.34 log$_{10}$ cfu/ml[1])
PO$_f$ (6.26 log$_{10}$ cfu/ml[1])
Slaughter and processing (No antimicrobial treatments /Air Chilling)
Slaughter and processing (Antimicrobial treatment ASC / Emersion Chilling with chlorination)
Slaughter and processing (Antimicrobial treatment ASC / Emersion Chilling with chlorination)
Slaughter and processing (No antimicrobial treatments / Air Chilling)
PO$_s$ (2.49 log$_{10}$ cfu/ml[1])
PO$_s$ (4.26 log$_{10}$ cfu/ml[1])
Chill Retail Distribution
Frozen Retail Distribution
PO$_r$ (2.49 log$_{10}$ cfu/ml[1])
Consumer handling and cooking of chicken
FSO (-4.45 log$_{10}$ cfu/g[2])
Chicken consumption

[1]per ml rinsate assuming testing by carcass rinse with100ml; [2]FSO per gram cooked chicken to meet the public health goal

Fig. 19.3 Summary of the illustrative example of a PO-led approach to controls in the chicken production chain from farm to fork

It is also clear from these illustrations that if there are events or practices that lead to situations where the assumptions used in this illustration are not valid, such as defeathering and evisceration contributing to increases in contamination levels (as shown in some studies), then these can have a significant impact on the PO values set.

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Appendix A: Glossary

Acceptance criteria (for a food operation) Statements of conditions that differentiate acceptable from unacceptable food operations.

Acceptance criteria (for lot acceptance) Statements of conditions that differentiate acceptable from unacceptable lots (batches) of food.

Control (verb) To take all necessary actions to ensure and maintain compliance with established criteria.

Control (noun) The state wherein correct procedures are being followed and criteria are being met.

Control measure Any action and activity that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Corrective action Any action to be taken when the results of monitoring at the critical control point indicate a loss of control.

Critical control point (CCP) A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Critical limit A criterion which separates acceptability from unacceptability.

Default criterion A conservative criterion established to ensure the safety of a food under worst-case conditions.

Deviation Failure to meet a critical limit.

Dose-response assessment (Codex) The determination of the relationship between the magnitude of exposure (dose) to a chemical, biological or physical agent and the severity and/or frequency of associated adverse health effects (response).

Expert panel A group of individuals who collectively have knowledge or experience with a hazard or food and the conditions that can lead to foodborne illness, and who have the ability to provide advice based on available scientific information.

Exposure assessment (Codex) The qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant.

Flow diagram A systematic representation of the sequence of steps or operations used in the production or manufacture of a particular food item.

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Food operation A location along the food chain where food is handled or prepared for commercial reasons.

Food safety objective (FSO) (Codex) The maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP).

Hazard (Codex) A biological, chemical, or physical agent in, or condition of, food with the potential to cause an adverse health effect.

Hazard analysis (in HACCP) The process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and therefore should be addressed in the HACCP plan.

Hazard Analysis Critical Control Point (HACCP) A system that identifies, evaluates, and controls hazards which are significant for food safety.

HACCP plan A document prepared in accordance with the principles of HACCP to ensure control of hazards that are significant for food safety in the segment of the food chain under consideration.

Hazard characterization (Codex) The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents which may be present in food. For chemical agents, a dose-response assessment should be performed. For biological or physical agents, a dose-response assessment should be performed if the data are obtainable.

Hazard identification (Codex) The identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods.

Monitor The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP in under control.

Performance criterion (PC) (Codex) The effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a PO or an FSO.

Performance objective (PO) (Codex) The maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption that provides or contributes to an FSO or ALOP, as applicable.

Process criteria The control parameters of a step, or combination of steps, that can be applied to achieve a performance criterion.

Product criteria A parameter of a food that can be used to assess the acceptability of a lot or consignment.

Qualitative risk assessment A risk assessment based on data which, while forming an inadequate basis for numerical risk estimations, nonetheless, when conditioned by prior expert knowledge and identification of attendant uncertainties permits risk ranking or separation into descriptive categories of risk.

Quantitative risk assessment A risk assessment that provides numerical expressions of risk and indications of attendant uncertainties.

Risk (Codex) A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.

Risk analysis (Codex) A process consisting of three components: risk assessment, risk management and risk communication.

Risk assessment (Codex) A scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization.

Risk Assessment Policy (Codex) Documented guidelines on the choice of options and associated judgements for their application at appropriate decision points in the risk assessment such that the scientific integrity of the process is maintained.

Risk characterization (Codex) The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment.

Risk communication (Codex) The interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Risk estimate (Codex) The quantitative estimation of risk resulting from risk characterization.

Risk management (Codex) The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options.

Risk profile (Codex) The description of the food safety problem and its context.

Sensitivity analysis A method used to examine the behavior of a model by measuring the variation in its outputs resulting from changes in its inputs.

Step A point, procedure, operation, or stage in the food chain including raw materials, from primary production to final consumption.

Tolerable level of risk (TLR) The level of risk proposed following consideration of public health impact, technological feasibility, economic implications, and that society regards as reasonable in the context of and in comparison with other risks of everyday life.

Uncertainty analysis A method used to estimate the uncdertainty associated with model inputs, assumptions and structure/form.

Validation (Codex) Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome.

Verification (Codex) The application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended.

Appendix B: ICMSF Publications

Books

Microorganisms in Foods 7: Microbiological Testing in Food Safety Management. 2nd edition.

Microorganisms in Foods 8: Use of Data for Assessing Process Control and Product Acceptance provides practical guidance on appropriate testing of food processing environments, processing lines, shelf life and finished product to enhance the safety and microbiological quality of the food supply. *Microorganisms in Foods 8* is intended for anyone using microbiological testing or engaged in setting microbiological criteria including government, food processors and the customers they supply. This book provides actionable information for food quality assurance professionals, food microbiologists, food technologist, veterinarians, public health workers and regulatory officials. *Springer. 1st edition, 2011, ISBN 978-1-4419-9373-1.* Available from Springer

Translations: Chinese, Japanese, Portuguese (ICFSM 2011. Microrganismos em Alimentos 8. Utilização de dados para avaliação do controle de processo e aceitação de produto. Blucher, São Paulo, Brazil), Spanish (ICMSF 2016. Microorganimos de los Alimentos 8. Uso de datos para evaluar el control del processo y la accpetación del producto. Editorial Acribia).

Microorganisms in Foods 7: Microbiological Testing in Food Safety Management. 1st edition**.** Continuing this excellent series, *Microorganisms in Foods 7* describes the role of microbiological testing in modern food safety management systems. It explores how risk assessment and risk management can be used to establish goals - known as "tolerable levels of risk" or "food safety objectives" – for use in controlling foodborne illness, and provides guidelines for establishing effective management systems to control specific hazards in foods. *Kluwer Academic/Plenum Publishers, 2002. ISBN: 0306472627.* Available from Springer

Translations: Chinese, Japanese, Spanish (ICMSF 2002. Microorganismos de los alimentos: análisis microbiológico en la gestión de la seguridad alimentaria. Editorial Acribia, SA, Zaragoza, Spain)

Microorganisms in Foods 6: Microbial Ecology of Food Commodities. 2nd edition. A second, further updated edition is published as New York: Kluwer Academic & Plenum Publishers, 2005. ISBN: 0-306-48675-X. Available from Springer.

Translation: Japanese

Microorganisms in Foods 6: Microbial Ecology of Food Commodities brings up to date the previous edition (*Microorganisms in Foods* 3: Vol 2) from 1980 taking account of developments in food

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processing and packaging, new ranges of products and foodborne pathogens that have emerged since then.

Translation: ICMSF (1998) Microorganismos de los alimentos: ecología microbiana de los productos alimentarios. Bernabé Sanz Pérez, José Fernandez Salguero, Manuel Ramis Vergés, Francisco León Crespo, Juan Antonio Ordoñez Pereda (translators), Editorial Acribia, SA, Zaragoza, Spain

Microorganisms in Foods 5: Characteristics of Microbial Pathogens is the only book to examine the characteristics of foodborne pathogens in relation to HACCP. *1996. London: Blackie Academic & Professional. ISBN: 041247350X. Available from Springer.*

Microorganisms in Foods 4: Application of the Hazard Analysis Critical Control Point (HACCP) System to Ensure Microbiological Safety and Quality was the first complete book devoted solely to the development and implementation of HACCP. *1988. Oxford: Blackwell Scientific Publications. ISBN: 0632021810. Also published in paperback under the title* ***HACCP in Microbiological Safety and Quality****, 1988, ISBN: 0632021810*

Translations: Japanese, Spanish ICMSF (1988) El sistema de análisis de riesgos y puntos críticos. Su aplicación a las industrias de alimentos, Malmenda PD and Garcia BM (translators), Editorial Acribia, Zaragoza, Spain

Microorganisms in Foods 3: Microbial Ecology of Foods brings food spoilage and health risks into sharper focus through its study of how different food processes, ingredients, and product characteristics affect the microflora of foods. *Vol. 1: Factors affecting life and death of microorganisms (ISBN: 0123635012). Vol. 2: Food commodities (ISBN: 0123635020). (1980). New York: Academic Press.* Vol. 2 updated as Microorganisms in Foods 6: (below).

Translations: ICMSF (1983) Ecología microbiana de los alimentos 1: factores que afectan a la supervivencia de los microorganismos en los alimentos, Burgos Gonzalez J et al. (translators), Editorial Acribia, Zaragoza, Spain

ICMSF (1984) Ecología microbiana de los alimentos 2: productos alimenticios, Sanz Perez B. et al. (translators), Editorial Acribia, Zaragoza, Spain

Microorganisms in Foods 2: Sampling for Microbiological Analysis: Principles and Specific Applications was the first comprehensive publication on statistically based sampling plans for foods. *2nd ed. (1986). Toronto: University of Toronto Press. ISBN: 0802056938.* Part 1 has been extensively revised and is now included in Book 7.

Translation: ICMSF (1981) Microorganismos de los alimentos 2: métodos de muestreo para análisis microbial ´ogicos: principios y aplicaciones especificas, Ordonez Pereda JA and Diaz Hernandez MA (translators), Editorial Acribia, Zaragoza, Spain

Microorganisms in Foods 1: Their Significance and Methods of Enumeration represented a major step in establishing a common understanding of, and developing standard methods for, important foodborne microorganisms. 1st ed. (1968) reprinted 1973, 1975 University of Toronto Press; *2nd ed. (1978), reprinted 1982, 1988 with revisions. Toronto: University of Toronto Press. ISBN: 0802022936.* Out of print.

Translations: Thatcher FS, Clark DS (1973) Microorganisms in foods 1: their significance and methods of enumeration [in Spanish: Garcia B. (translator)], Editorial Acribia, Zaragoza, Spain

Selected Publications and Reports

Cordier, J.L. and the International Commission on Microbiological Specification for Foods (2013) Microbiological Criteria and Indicator Microorganisms. In Food Microbiology: Fundamental and Frontiers. M.P. Doyle and R.L. Buchanan, eds. ASM Press, Washington DC pp.81–90.

International Commission on Microbiological Specifications for Foods (2013) Usefulness of testing for *Clostridium botulinum* in powdered infant formula and dairy-based ingredients for infant formula. Dated: 27 August 2013 (https://search.yahoo.com/yhs/search;_ylt=A0LEVitzRMVZ9RMAwjInnIlQ;_ylc=X1MDMTM1MTE5NTY4NwRfcgMyBGZyA3locy1tb3ppbGxhLTAwMwRncHJpZAMyUzRwaXZOa1RtbUhnamd5MTBWVG9BBG5fcnNsdAMwBG5fc3VnZwMwBG9yaWdpbgNzZWFyY2gueWFob28uY29tBHBvcwMwBHBxc3RyAwRwcXN0cmwDMARxc3RybAMxMQRxdWVyeQNTUkMlMjBJQ01TRgR0X3N0bXADMTUwNjEwMDc5MA--?p=SRC+ICMSF&fr2=sb-top&hspart=mozilla&hsimp=yhs-003.

Zwietering, M.H., Stewart, C.M., Whiting, R.C., International Commission on Microbiological Specifications for Foods (ICMSF) (2010) Validation of control measures in a food chain using the FSO concept. Food Control 21, 1716–1722.

International Commission on Microbiological Specifications for Foods (ICMSF). (2009) "Relating Microbiological Criteria to Food Safety Objectives and Performance Objectives". van Schothorst, M., Zwietering, M., Ross, T., Buchanan B., Cole, M. Food Control, 20:967–979

International Commission on Microbiological Specifications for Foods (ICMSF). (2006) "A Simplified Guide to Understanding and Using Food Safety Objectives and Performance Objectives. Available at www.icmsf.org in multiple languages.

International Commission on Microbiological Specifications for Foods (ICMSF) (2004) Papers at the 36th annual meeting of the Swiss Society for Food Hygiene (SGLH) in October 2003. The papers have been published as communications in the journal *Mitteilungen aus Lebensmitteluntersuchung und Hygiene*, Volume 95, Issue 1, 2004. They can be downloaded below for personal use. Copyright remains with the journal.

Buchanan, B. Principles of risk analysis as applied to microbial food safety concerns
Cole, M. Food safety objectives - concept and current status
Gorris, L. Performance objectives and performance criteria - two sides of the food chain
Cordier, J.-L. Microbiological criteria - purpose and limitations
Dahms, S. Microbiological sampling plans - statistical aspects
Tompkin, B. Environmental sampling - a tool to verify the effectiveness of preventive hygienic measure
Pitt, J. Application of the food safety objective concept to the problem of aflatoxins in peanuts
Gram, L. How to meet an FSO - Control of Listeria monocytogenes in the smoked fish industry

International Commission on Microbiological Specifications for Foods (ICMSF) [M. van Schothorst, Secretary]. (1998) "Principles for the establishment of microbiological food safety objectives and related control measures". Food Control, 9(6):379–384

International Commission on Microbiological Specifications for Foods (ICMSF). (1998) "Potential application of risk assessment techniques to microbiological issues related to international trade in food and food products". J. Food Protection, 61(8):1075–1086

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International Commission on Microbiological Specifications for Foods (ICMSF). (1994) "Choice of sampling plan and criteria for *Listeria monocytogenes*". Int. J. Food Microbiol.22:89–96.

Tools

Microbiological Sampling Plan Tool (http://www.icmsf.org/main/software_downloads.html)

Control Measures (FSO) Validation Tool (http://www.icmsf.org/main/software_downloads.html)

Appendix C: Meetings Sponsors 2010 to 2016

ICMSF General Conference Sites and Major Sponsors

No.	Year	Location	Sponsors
43	2010	Annecy, France	Members' agencies; Fondation Marcel Mérieux; ICMSF sustaining fund
44	2011	Melbourne, Australia	Members' agencies; Australian Institute of Food Science and Technology; Commonwealth Scientific and Industrial Research Organisation (CSIRO); Ecolab Inc.; Food Safety Authority of Ireland (FSAI); Nestle; Unilever; ICMSF sustaining fund;
45	2012	Xiamen, China	Members' agencies; Chinese Institute of Food Science and Technology; Commonwealth Scientific and Industrial Research Organisation (CSIRO); Food Safety Authority of Ireland (FSAI); Nestle; Unilever; Universidade de Sao Paulo; ICMSF sustaining fund
46	2013	Dubai, United Arab Emirates	Members' agencies; Dubai Municipality; Food Safety Authority of Ireland (FSAI); Commonwealth Scientific and Industrial Research Organisation (CSIRO); Nestle; Pepsico; Unilever; Universidade de Sao Paulo; ICMSF sustaining fund
47	2014	Iguazu, Brazil	3 M; BCN; Biomeriex; Cargill; Congreso Latinamericano de Microbiologia e Higiene de Alimentos; Du Pont; (Life, Oxoid) Eurofins; Food Safety Authority of Ireland (FSAI); Interlab; JBS; Laboratório, SP, Biorad; Mondelez; Neogen; Nestle; Pepsico; Qjagem; Solabia Biotec; Sovereign; Romer Labs; Thermo; Unilever; Universidade de Sao Paulo; ICMSF sustaining fund
48	2015	Tokyo, Japan	Members' agencies; Commonwealth Scientific and Industrial Research Organisation (CSIRO); Food Safety Authority of Ireland (FSAI); Food Safety Commission of Japan; Ikari Corporation; International Life Sciences Institute Japan (ILSI, Japan); Japanese Society of Food Microbiology (JSFM); Kao Corporation; Legend Applications Inc.; Meiji Co., Ltd. R&D Division; Mitsubishi Research; Mondelez; Nestle; Nichirei Corporation; Nisshin Seifun Group Inc.; Pepsico; Suntory World Research Center; Tsukuba Food Evaluation Center Co., Ltd; Unilever; Universidade de Sao Paulo
49	2016	Penang, Malaysia	Members' agencies; Commonwealth Scientific and Industrial Research Organisation (CSIRO); Food Safety Authority of Ireland (FSAI); International Association for Food Protection; International Life Sciences Institute Southeast Asia; Mondelez; Nestle; Pepsico; Unilever; Universidade de Sao Paulo; ICMSF sustaining fund

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Index

A
Acceptable level of risk, 33
Acceptance criteria
  application, 108–109
  approval of suppliers in business-to-business relationships, 109–111
  auditing food operations, 113–115
  chemical and physical, 340, 377, 399, 426
  commercial buyers, 105
  country-to-country food product flows, 105
  equivalence, 106
  guidelines, 107, 108
  lot acceptance, 112, 113
  microbiological, 339, 398, 399, 426
  organoleptic, 340, 376, 399, 426
  parameters, 107
  samples, 105
  specifications, 108
  standards, 107
Acceptance/rejection, sampling plans, 140
Acidified sodium chlorite (ASC), 19
Adenosine tri-phosphate (ATP) testing, 121
Aerobic plate counts (APC), 320
Aeromonas hydrophila, 169
Aflatoxins
  acute human aflatoxicosis, 330
  ALOP/FSO, 336
  CCFAC and CCCF, 333
  chemical contaminants, 330
  chemical metabolites, 329
  constructed sampling plans, 330
  distribution/marketing, 336
  dose response analysis, 330
  exposure assessment, 331, 332 (*see also* Food safety systems)
  in food supplies, 330
  genotoxic and carcinogenic compounds, 331
  governmental regulations, 333
  hazard identification and characterization, 330
  high performance liquid chromatography, 329
  immunochemical methods, 329
  mutagenic and carcinogenic substances, 330
  NOAEL, 333
  normal food processing, 334 (*see also* Performance criteria)
  PO (*see* Performance objectives (PO))
  preparation/consumption, 336
  primary *Aspergillus* spp., 329
  primary production
    increase during storage ($\sum$I), 335
    initial level of contamination (Ho), 334
  public health interventions, 334
  reduction in levels, processing ($\sum$R), 335
  risk characterization, 333
  sub-optimal field practices and poor storage conditions, 334
  types, 329
  See also Acceptance criteria
Air sampling, 202
Alicyclobacillus spp., 272
ALOP/food safety
  annual incidence, listeriosis, 368
  GHP and HACCP programs, 369
  public health goal, 369
  WHO/FAO risk assessment, 369
  "zero tolerance" policy, 369
Amnesic shellfish poisoning (ASP), 16
Analytical units
  dilution and homogenization, 204
  withdrawing sample, 204
Antimicrobial additives, 367, 368
Antimicrobial resistance, 437
Antimicrobial treatments, 79
Appropriate level of protection (ALOP), 1, 3, 4, 7, 21, 23, 33, 63, 106
Approval of suppliers
  in business-to-business relationships
    approval procedures, 110–111
    FSO/PO role, 109–110
Assessing equivalency
  food safety management systems, 101

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International Commission on Microbiological Specifications for Foods (ICMSF),
Microorganisms in Foods 7, https://doi.org/10.1007/978-3-319-68460-4

Assessment of process control, 24
Attributes plans
  microorganisms concentration, 156
  three -class, 147, 150, 157, 159
  two-class, 145–147, 156, 159
Attributes sampling plans
  quantitative performance, 93
Auditing
  food operations, supplier acceptance
    FSO/PO, 114, 115
    internal audits, 114
    Plan-Do-Check-Act, 114
    pre-determined scheme, 113
    principles, 114
    standardization of audit activities, 114
    supplier audits, 114
Average outgoing quality (AOQ), 252
Average outgoing quality level (AOQL), 252
Average run length (ARL), 254
Average sample number (ASN), 252

B
Bacteria
  botulism, 15
  Campylobacter, 14
  Cronobacter spp., 14, 15
  E. coli, 14
  L. monocytogenes, 14
  norovirus, 13
  salmonellae, 13
  Shigella spp., 14
Biased sampling, 241
Binomdist, 136
Binomial distribution
  acceptance, 136
  computer spreadsheet software, 136
  defective units, 136–138
  definition, 136
  description, 135
  microbiological testing, 135
  OC-curve, 134, 136
  probability of acceptance, 136
  proportion of defective units, 136
  sample units, 135
  sampling plans, 137
  sampling schemes, 137, 138
Biofilms, 275, 283
Biological hazards, 66
Bivalve molluscs, 16
Bivalves
  ALOP/FSO and consumer protection, 423, 424
  characteristics, 411
  epidemiology, 413
  exposure assessment, 415–418 (*see also* Food safety systems)
  foodborne outbreaks, 411
  HAV, 412
  high quality animal proteins, 411
  human disease transmission, 411
  NLVs, 412
  pathogenic microorganisms, 411 (*see also* Performance criteria)
  post-harvest control (*see* Post-harvest control)
  pre-harvest control, 419–421
  real-time RT-PCR methods, 419
  relaying and depuration, 412
  risk characterization, 418, 419
  risk communication and education, 423
  single-cell algae, 411
  wastewater treatment system, 419
  See also Acceptance criteria
Botulism, 15
Brucellosis, 70
Business deal, 105
Business-to-business relationships
  approval of suppliers
    approval procedures, 110–111
    FSO/PO role, 109

C
Campylobacter, 14, 19
  antimicrobial resistance, 437
  bacterial enteric human infections, 435
  campylobacteriosis in humans, 436
  chicken production farms, 435
  consumer protection, 443
  distribution and retail sale under chill/frozen conditions, 435
  dose-response models, 437, 438 (*see also* Exposure assessment)
  flagella-mediated motility, 437
  FSO, 443, 444
  gastroenteritis cause in Europe, 436
  GBS, 436
  local complications, 437
  non-spore forming, gram negative spiral-rod shaped bacteria, 436
  optimum growth temperature, 436 (*see also* Performance objectives (POs))
  poultry consumption, 436
  public health authorities, 444, 445
  risk characterization, 442–443
  wild and domesticated birds and mammals, 436
Campylobacter Performance Target, 69
Campylobacter spp., 273
Campylobacteriosis, 14
Certificate of Analysis (COA), 120, 298
Chemical hazards, 66
Chicken meat, *see Campylobacter*
Ciguatera, 16
Codex Alimentarius, 105, 106
Codex Alimentarius Commission (CAC), 1, 4, 5, 10, 12, 117–119, 221

Codex Alimentarius Committee on Food Additives and Contaminants (CCFAC), 333
Codex Alimentarius Standard, 352
Codex Committee on Contaminants in Foods (CCCF), 333
Codex Committee on Food Hygiene (CCFH), 10, 12, 13
Codex framework, 22
Codex system, 3
Coding system, 140
Combined process, 345
Commercial buyers, 105
Conceptual equation, 82, 83
Consumer protection, 32, 64, 70
Consumer risk, 134, 137, 138
Consumption level, 19
Containers, 199
Contamination
  prevention, 263
Control authorities, 108, 109
Control charting software, 323
Control charts
  APC, 320
  binomially distributed data, 317, 320
  F Chart, 322, 323
  Poisson distributed characteristics, 317
Control measures
  assessing equivalency of food safety management systems, 101
  consumer protection, 64
  default values, 95
  definition, 64
  E. coli O157:H7, 100–101
  effectiveness, 70–79
  FBOs, 63
  food manufacturers and food business operators, 64
  food preparation and consumption stage, 65
  food supply chain, 64
  foodborne diseases, 70–75
  FSOs/POs
    establishment, 69
    FBOs, 68
    food chain, 69
    food products/categories, 68, 69
    government and stakeholders, 68
    MC, 69
    position, 69
    skills and resources, 68
    stakeholder interactions, 68
  initial levels, 64
  livestock, 70
  MC, 64
  microbiological sampling, 92, 93
  microbiological testing, 64
  monitoring and verification, 99
  PC (*see* Performance criterion (PC))
  prerequisite programs, 63
  preventing increase of levels, 64
  process criteria, 92
  process validation, 95–98
  product category, 64
  product criteria, 92
  quantitative performance, attributes sampling plans, 93–95
  reducing levels, 64
  trichinosis and typhoid fever, 70, 76
  UK Advisory Committee on the Microbiological Safety of Foods, 99–100
Cost-benefit analysis, 37
Country-to-country food product flows, 105
Critical control points (CCPs), 19, 66, 67, 108, 115, 121, 122, 263
Cronobacter spp., 14, 15, 270
Cumulative sum (Cusum) charts, 305

D
Data collection
  food operations, 97
Default criteria, 23
Default values
  control measures, 95
Defective units, 136–138
Degree of variability
  "cross-batch testing", 298
  end product testing, 298
  equipment and environmental tests, 298
  equipment maintenance and performance, 299
  food manufacturers, 297
  food processing parameters, 299, 300
  ingredient data, 298
  in-line sampling, 298
  "microbiological history", 297
  process and/or quality manager, 297
  seasonal and weather effects, 299
  shelf life sampling, 299
  solid foods, 299
Depuration process, 420
Destroying pathogens, 64
Deterministic/point estimate risk assessments, 55
Diarrhetic shellfish poisoning (DSP), 16
Discrete events, 82, 85, 90, 91
Discrimination
  OC function, 138
DNA extraction procedures, 234
Domoic acid poisoning, 16
Dose-response models
  adjustment factors, 363
  hazard characterization, 362
  healthy individuals and high-risk populations, 362
  host variability, 362, 363
  Internalin A gene mutation, 362
  L. monocytogenes risk assessment, 363
  serovars, 362
  strain variability, 363
Dry-mix process, 345

E
E. coli O157:H7, 100, 223, 271
Effectiveness of control measures
  antimicrobial treatments, 79
  carcasses and in ground meat, 78, 79
  commercially prepared shelf-stable foods, 70, 78
  foodborne disease, 70
  non-typhoid salmonellae, 79
  performance standards, 78, 79
  prevalence rate, 79
  Shigellae, 78, 79
  trichinellae, 77
  trichinosis, 70, 76
  typhoid fever, 77, 78
  USDA-FSIS, 79
Egg products, MC, 127, 128
End-product testing, 131, 298
Enrichment procedures
  E. coli O157:H7, 223, 224
  LOD, 221
  target microorganism
    analytical units, 223, 224
    cultural and non-cultural detection, 224
    genomic techniques, 224
    and incubation period, 224, 225
    injured cells, 227
    L. monocytogenes, 225, 226
    non-injured cells, 225
EnterNet/FoodNet, 39, 41
Enterobacteriaceae, 220
Enterohaemorrhagic *E. coli* (EHEC), 14, 169, 388, 390–393, 395, 396
  ALOP/FSO, 394, 395
  consumer storage, 394
  contaminated agricultural water, 386
  control of pathogens, 386
  cross-contamination, 402, 403
  deterministic approaches, 400
  distribution/marketing, 393
  dose response, 388–390
  efficacy of disinfection, 401
  exposure assessment (*see* Exposure assessment)
  foodborne infection outbreaks, 386
  global production, 385
  growth of, 386, 401, 402
  hazard identification, 387
  holistic/systems approach, 385
  hygiene controls, 385
  O157:H7, 388 (*see also* Performance criteria)
  PO (*see* Performance objectives (PO))
  primary production and processing, 393
  public health improvement, 385
  risk assessments/risk profiles, 386–387
  risk characterization, 393
  RTE leafy vegetables, 388
  Salmonella enterica, 386
  stochastic approaches, 403–406
  syndromes, 388
  testing, 402
  washing prior to consumption, 394
Enteroinvasive *E. coli* (EIEC), 169
Enteropathogenic *E. coli* (EPEC), 169
Environmental sampling
  data management, 281
  electrostatic wipes/roller, 276
  investigational sampling
    contamination detection, 281, 283
    L. monocytogenes, 281–283
    S. aureus, 283
    Salmonella, 281, 282
  monitoring programs, 280
  number, frequency and timing, 279
  processing systems, 279
  routine sampling program, 276
  tools and techniques, 276
  usage, 275
  zone concept, 277, 278
Equipment and environmental tests, 298
Equivalence
  acceptance criteria, 106
Escherichia coli O157:H7, 19
Estimated probability, 132
European Rapid Alert System for Food and Feed (RASFF), 413
Evaluation of risk
  competent authorities, 31
  consumer protection, 32
  control, 31
  control of hazard, 36
  differences, 36
  epidemiologic data
    articulation, 38
    EnterNet/FoodNet, 39, 41
    food source and conditions, 42
    foodborne disease, 39, 41
    laboratory testing, 42
    levels, 38
    non-typhoidal human *Salmonella* cases, 39, 40
    policy, 42
    quantitative risk assessment, 39
    scope, 38, 39
    surveillance data collection, 41
    surveillance systems, 39, 41
  expert panels, 44, 45
  FBOs, 35
  food control agencies and industry, 43
  food safety status, 42
  foodborne listeriosis, 32, 33
  FSOs/POs (*see* FSOs/POs)
  functions, risk manager, 44
  guidance, 42
  ICMSF, 34
  identification, food safety problem, 43, 44
  knowledge, 33
  level of protection, 31, 32
  management of microbial hazards, 43
  managers, 31
  measures, 43
  microbiological risk assessment, 35
  principles of risk analysis, 43

public health, 33
qualitative/(semi-)quantitative risk assessment (*see* Qualitative/(semi-)quantitative risk assessment)
quantitative risk assessment, 35
risk-management option, 34
safety assessments, 43
TLR (*see* Tolerable level of risk (TLR))
WGS techniques, 33
WTO, 33
WTO/SPS agreement, 43
Expert panels, evaluation of risk, 44, 45
Export certificate, 111
Exposure assessment
actual liver cancer, 332
actual/anticipated human, 360
biomarkers, 331
CEN, 418
characteristics, behavior and survival, 417
chicken slaughter, 439, 440
consumer practices and consumption, 416
contaminated foods, 331
cooked meat processing, 361
cross contamination, home preparation, 442
description, 360
deterministic and probabilistic models, 331
groundnut consumption, 332
HBV and HCV, 332
HCC, 332
home cooking, 441, 442
hygiene control and cross-contamination, 390, 391
JECFA, 332
on-farm biosecurity and infection, 439
oysters contamination, 415, 416
post-cooking exposure and contamination, 361
predictive models, 391
pre-harvest contamination, 390
pre-harvest control, 421
product–predictive models, 361
raw materials, 361
Exposure assessment (*cont.*)
retail storage and distribution, 441
RTE meats, 361
RT-PCR and RT-qPCR, 417
seasonal influence, 416
shellfish, 418
time and temperature, supply chain, 362
time, temperature and potential growth, 391–393
USDA monitoring program, 361
viral hepatitis, 332
virus prevalence, 416

F

Failure Control Chart (F Chart), 322, 323
FAO/WHO, 4
Farm management practice
A. flavus infection, 340
aflatoxins in peanuts, 340
nontoxigenic spores, 341
threshing and mechanical drying, 341
weed control, 340
First party audits, 114
Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) food standards program, 10
Food business operators (FBOs)
evaluation of risk, 35
food safety management system, 35
Food business, MC
food operators, 121
GHP, 121
HACCP, 121
regulatory standards, 121
Food class, 180
Food contact surfaces (FCS), 378
Food operations
data collection, 97
Food operators, 21
Food processing, 20
Food safety control systems, 2
Food safety management system
ALOP, 7
assessing equivalency, 101
concepts, 10
definitions, 5
development, 10–13
FSO, 7, 9
HACCP systems, 9
hazard-food combination, 7
industries sectors/FBOs, 8
industry and government, 6
MC, 9
microbiological testing, 24, 25
PC, 9
POs, 8, 9
prerequisite programs, 9
processing/production operation, 9
risk managers, 6, 7
rough risk assessment/risk profile, 7
system thinking, 9
Food safety objectives (FSOs)
and ALOP, 4, 7
CAC, 4, 5
Codex Committee, 4
concept, 3, 4
development, 4
dose-response relationships, 4
enhancement program, 4
epidemiological evidence, 5
establishment, 4
food safety management system, 4
ICMSF, 4
maximum frequency, 7
microbiological criteria, 5
and PO, 1, 8
risk assessment, 4
risk characterization, 4

Food safety systems
GAPs, 406
GHP programs, 340, 341, 353, 378, 406, 427
HACCP programs, 340, 353, 377, 406, 427
microbiological criteria, 353
regulatory requirements and criteria, 378, 379, 406, 407, 427
Food/hazard combination, 3
Foodborne Disease Burden Epidemiology Reference Group (FERG) project, 18, 32
Foodborne diseases
control measures, 70–75
Foodborne hazards, 10, 11
Foodborne illness
bacteria, 13–15
estimation, 32
FSOs/POs, 21
protozoa, 16
seafood toxins, 16
surveillance and trends, 17–18
toxigenic fungi, 17
viruses, 15–16
Foodborne listeriosis, 32, 33
Fresh-cut RTEs, *see* Enterohemorrhagic *E. coli* (EHEC)
FSOs/POs
concepts, 36
control measures (*see* Control measures)
definitions, 63
evaluation of risk
articulation, 48
CAC, 48
competent authorities, 47, 48
concentration/prevalence, 47
concepts, 46, 49
control authorities, 49
epidemiologic data, 50
establishment, 50
expert panel, 50, 51
FBOs, 49
food control systems, 45
food hazard, 46
governments and food industries, 45
guidelines, 45
hypothetical examples, 46
industry, 48, 49
investigation, foodborne disease, 49
level of control, 47
microbial hazard, 46
pathogen-product, 49
purpose, 48
quantitative and verifiable, 47
risk-based metrics, 47
value(s), 47
WTO/SPS agreement, 50
food safety, 21
food safety management, 21–22
GMP/GHP and HACCP, 57
government risk managers, 58
hazard-food combinations, 57
pathogen-food, 57
qualitative/(semi-)quantitative risk assessment, 56
reduce foodborne illness, 21
stringency, 57
values, 57
Fumonisins, 17

G

General Principles of Food Hygiene, 264
Genomic based methods, 234
Geometric and arithmetic means, 187, 188, 190, 191, 193
GHP programs, 353
Global Food Safety Initiative (GFSI), 110
Global trade, 105
Good agricultural practices (GAP), 1, 334
Good hygiene practices (GHP), 1, 131, 183, 263
application, 63, 65
components, 65, 66
farm management practice, 340, 341
MC, 121
outbreaks, 66
prerequisite programs, 65, 66
shelling plants, 341
Good manufacturing practices (GMPs), 1, 154, 160, 161
GRAS (generally regarded as safe) status, 17
Group of units, 132
Guillain-Barré Syndrome (GBS), 171

H

HACCP programs, 353
HACCP-based system, 3
Handling procedure, 127
Hazard analysis and critical control points (HACCP)
and prerequisite programs, 20, 63, 68
CCPs, 66, 67
food manufacturers design processes, 67
FSO/PO, 67
ICMSF and Codex Alimentarius, 67
MC, 121–122
principles, 66, 67
production of safe food/food ingredients, 67
regulatory authorities, 67
risk management principles, 67
system and guidelines, 66
Hazard characterization, 362
Hazard identification, 52, 387
Hazard-food combinations, 49
Heat-treated food risk, 179
Hemolytic uremic syndrome (HUS), 388
Hemorrhagic colitis, 388
Hepatitis A virus (HAV)
case-fatality rate, 415
epidemic and sporadic fashion, 413
genotypes, 412
incubation period, 415

Picornaviridae family, 412
public health problem, 413
vaccination control, 423
virus' infectivity, 415
VP1X2A and 2C regions, 415
Hepatitis B virus (HBV), 332
Hepatitis C virus (HCV), 332
Hepatocellular carcinoma (HCC), 332, 333
High hydrostatic pressure (HP), 422
High-pressure processing (HPP), 375
Histamine poisoning, 168
Histamine/scombroid poisoning, 16
Histo-blood group antigens (HBGAs), 414
Home-canned/home-prepared foods, 15
Homogeneous distribution, 92
Homogenisation, 198
Host variability, 362, 363

I
ICMSF spreadsheet tool, 449
ICMSF tool, 195
Immunomagnetic concentration systems, 232–234
Indicator organisms, 124, 125
Injured cells, recovery
pre-enrichment conditions, 205
Salmonella, 205
selective agents, 206
time spans, 205
In-line sampling, 298
In-pack pasteurization, 367
In-package pasteurization, 371
Internal audits, 114
International commerce, 22
International Commission on Microbiological Specifications for Foods (ICMSF/Commission), 1
International non-governmental observer (INGO), 13
International standards, 110
Investigational sampling, 244
ISO/IEC 17021:2011 (ISO 2011b), 114

J
Jameson effect, 229–231
Joint FAO/WHO Expert Committee on Food Additives (JECFA), 332
Joint FAO/WHO Meetings on Microbiological Risk Assessment (JEMRA), 12

L
Laboratory challenge tests
process validation
composition of food, 96, 97
conditions of storage, distribution and preparation, 97
intrinsic resistance of pathogen, 96
non-pathogens, 96
Laboratory information management systems (LIMS), 203
Laboratory quality assurance, *see* Quality assurance
Level of protection, 31, 32
Limit of detection (LOD), 219
Liquid samples, 201, 202
Listeria innocua, 231, 232
Listeria monocytogenes
ALOP/food safety, 368, 369
antimicrobial additives, 367, 368
brine chiller, 358
Canadian outbreaks, 358
CDC, 364
consumer storage, 368
cooked meats, 357
cooking, 365
deli-meats, flow chart, 365
distribution/marketing
cross-contamination at retail, 368
time and temperature control, 368
dose-response models (*see* Dose-response models)
estimated incidence, listeriosis, 364
exposure assessment, 360, 362 (*see also* Food safety systems)
2003 FDA/FSIS risk ranking model, 363 (*see also* Acceptance criteria)
foodborne listeriosis prevention, 364
foodborne pathogen, 357
GHP and HACCP, 365
heating prior to consumption, 368
independent epidemiological data, 363
in-pack pasteurization, 367
microbial hazard, 357
NAFSS, 364
outbreaks since 2009, 358, 359 (*see also* Performance criteria)
PO (*see* Performance objectives (PO))
primary production, raw materials handling, 365
recontamination, cooking and packaging, 365–367
risk assessments/risk profiles, 358–360
temperature, time and humidity, 358
vulnerable populations, 368
Lot, 140
Lot acceptance, 112, 113
Low-acid canned foods, 19

M
Microbial contamination
in distribution, retail and consumer, 268
outbreaks
dehydrated dairy products, 266
foodservice operations, 266
L. monocytogenes, 268
product and pathogen, 267
Salmonella enterica, 268
pathogen concentration, 266
primary production, 265
ready-to-eat foods, 266

Microbial hazards
eating customs, 180
life threatening, 171–178
moderate, 170, 171, 173
serious, 171, 173
type, 180
Microbial population, 126
Microbiological criteria (MC)
applications, 34, 118, 119
CAC, 118
components, 123–127
control measures, 64, 69
for *Cronobacter* spp., 117
definition, 119
egg products, 127, 128
establishment, 117, 122, 123
food business, 121–122
and FSOs/POs, 118
indicator, 166, 167
microbiological guideline, 119, 120
microbiological specification, 119, 120
microbiological standard, 119
pathogens, 167
principles, 117
quantifiable risk reduction, 117
regulatory authorities, 120, 121
reporting, 127
types, 117
unquantifiable, 117
utility, 166
Microbiological guideline, 119, 120
Microbiological hazards
food safety management system (*see* Food safety management system)
food safety metrics, 1
HACCP approaches, 2
HACCP principles, 1
ICMSF/Commission), 1
microbiological testing, 2
product criteria, 2
RA, 1
risk-based metrics, 3–6
Microbiological limits, 19, 125, 126
Microbiological method, 127
Microbiological risk assessment (MRA), 51
Microbiological sampling, 202
and PC, 92, 93
Microbiological specification, 119, 120
Microbiological standard, 119
Microbiological testing
contamination
lot/batch, 209, 210
retesting, 210
Salmonella, 210
isolation and identification, 220
issues, 206
non-random distributions, 207–209
retesting, 206
Microorganisms, 123–125
Modified atmosphere packs (MAP), 368
Monitoring and verification, control measures, 99
Most probable number (MPN), 210
"Moving Range" (MR) charts, 314
Moving window approach, 69
Moving windows
Campylobacter, 256
Salmonella, 256, 257
Murine norovirus (MuNoV), 417
Mycotoxins, 17

N
Neurotoxic shellfish poisoning (NSP), 16
Niches, 275, 283
No Observed Adverse Effect Level (NOAEL), 333
Non-conforming sample units, 132, 136
Non-food contact surfaces (NFCS), 378
Non-random distributions, 207–209
Non-tariff trade barriers, 117
Non-typhoid salmonellae, 79
Non-typhoidal gastroenteritis, 13
Norovirus (NoV), 13, 15, 16
Norwalk/Norwalk-like viruses, 15
Norwalk-like viruses (NLVs)
commercial production areas, 415
complications, 414
dose-response curves, NoV, 414
FUT-2, 414
genetic factors and acquired immunity, 414
genogroups, 412
NoV genotypes, 414
person-to-person transmission, 414
projectile vomiting, 414
recombinant NoV strains, 412
self-limited illness, 413
small round-structured viruses, 412
zoonotic transmission, 412
Nucleic acid-based analytical methods, 234

O
Objective basis of comparison, 106
Ochratoxin A, 17
On-line reprocessing (OLR), 19
Operating characteristic (OC) curve, 134, 235
Operating characteristic (OC) function
binomial distribution, 135–138
consumer risk, 134, 137, 138
discrimination, 138
OC-curve, 134
producer risk, 134, 137, 138
stringency, 138–139
Operational prerequisite programs (OPRPs), 353
Osmotic shock, 205
Out of control action plan (OCAP), 295

P
Packaging material, 200
Paralytic shellfish poisoning (PSP), 16
Passive notification systems, 39
Pathogen environmental monitoring programs, 280
Pathogen testing, 281
Pathogenic microorganisms, 96
Pathogens
  clinical features, 169, 170
  diagnosis, 170
  ecological features, 168, 169
  epidemiologic considerations, 167–168
Pathogens control
  biofilms, 275
  food processing, 273, 274
  macro and micro design, 274
  microbial colonization, 274
  niche, 275
Peanuts, *see* Aflatoxins
Performance criterion (PC)
  color sorting, 338
  control measures, 424
    additions/reductions, 82
    ALOP and FSO, 80
    conceptual equation, 82, 83
    cross-contamination/independent contamination, 90
    definition, 80
    discrete events, 90, 91
    food supply chain, 81
    growth and recontamination, 82
    guidance, 82
    guidelines, 81
    levels of reduction, 89
    mean log and standard deviation values, 89
    microbiocidal/microbiostatic, 80
    outcome, 81
    and PdC, 80
    performance standards, 81
    prevalence/concentration, 80
    probability distribution of initial cell level, 87, 89
    public health protection, 80
    recontamination/dependent contamination, 90
    safe product and process design and implementing, 80
    traditional food safety metrics, 80
  food-borne infections, 425
  HAV levels reduction, 426
  hazards removal, 424
  initial levels control, 425
  and microbiological sampling, 92, 93
  NoV levels reduction, 426
  personal hygiene practices, 426
  post-package pasteurization, 374
  process criteria, 338, 339, 397 (*see also* Process criteria)
  product criteria, 339, 376, 398
  re-contamination prevention, 425
  shelf-life limits, 339, 376, 398
Performance metrics, 36
Performance objectives (POs)
  consumer handling and cooking practices, 445–449
  control measures, FSO, 370
  cooking effect, 446
  cooking recontamination, 370, 371
  cross-contamination effect, 446–449
  description, 369
  distribution/marketing, 337, 395, 396
  dose-response relationships, 395
  food processors, 395
  FSO, 337
  ingredients, 337, 395
  in-package pasteurization, 371
  manufacturing/port of entry, 337, 396
  PO-led approach, 453
  preparation/ingredients, 373, 374
  primary production, 337, 338, 396
  retail distribution, control measures, 449–451
  risks, 370
  slaughter and processing, 451, 452
  storage time and temperature, 371, 373
  time of consumption, 336
Plan-Do-Check-Act, 114
Plesiomonas shigelloides, 169
Poisson distribution, 197
Polymerase chain reaction (PCR), 213, 244
Population, 132
Population probability, 132
Post-harvest control
  handling and hand washing practices, 422
  heat treatment/cooking, 421
  HP, 422
  ionizing radiation, 421
  refrigeration and freezing, 422
Post-pack pasteurization, 361
Powdered infant formula (PIF)
  aerobic mesophilic bacteria, 346
  chemical, physical and nutritional, 352
  control measures
    contamination routes, 348
    distribution/marketing, 349
    manufacturing/processing, 348, 349
    preparation/handling, 349–351
    primary production, 348
  exposure assessment, 347
  FSO/PO, 351
  hazard characterization, 347
  hazard identification, 346
  health effects, 345
  hygiene indicator, 346
  microbiological safety and quality, 350, 352, 353
  new medical research, 345
  organoleptic, 352
  performance criterion, 352
  PO
    distribution, 351
    manufacturing, 351
    preparation, 351
    primary production, 351
  risk characterization, 348
  wet-mix, dry-mix and combined, 345

Pre-harvest contamination, 390
Pre-harvest control
  relaying and depuration, 420, 421
  sewage contamination prevention, 419, 420
Prerequisite programs, 63, 264
  and HACCP, 20
Preservation conditions, 179
Prevalence probability, 132
Probabilistic risk assessments, 55
Probabilities of acceptance, 148, 151, 158, 185, 186
Probability, 131–132
  density curve, 136
  lot acceptance, 135
Process capability study
  CCP values, 301
  data, 301
  description, 300
  HACCP plan, 300
  statistical process control methods, 301
  temperature of pasteurization, 301
  variability and error, 302, 303
Process control, 24
Process criteria
  distribution/marketing, 397
  HPP, 375
  processing, 397
  thermal processes, 374, 375
Process validation
  control measures
    data collection, food operations, 97
    data development, 96
    laboratory challenge studies, 96–97
    product quality/reduced cost, 95
    strengths and weaknesses, 96
    variability, 97, 98
Process variability, 97, 98
Producer risk, 134, 137, 138
Product criteria, 1, 2, 23, 92, 107, 339, 376, 398
Product unit size, 134
Proficiency testing, 214
Protozoa, 16
Psychrotrophic pathogens, 15
Pulsed-field gel electrophoresis (PFGE), 270
Purchase specifications, 108

Q
Qualitative/(semi-)quantitative risk assessment
  establishment of FSO/PO, 56
  evaluation of risk
    assumptions, 52
    expert panels, 51
    exposure assessment, 52
    hazard characterization, 53
    hazard identification, 52
    mathematical approaches, 55
    MRA, 51
    purpose, 51
    risk characterization, 54, 55
    risk estimate, 52
Quality assurance
  laboratory accreditation, 212
  PCR, 213
  proficiency testing, 214
  programs, 212
  validation, 213
Quality errors
  diluent volume, 211
  false positive and negative, 212
  laboratory sampling, 211
  pipette volume, 211
Quality objectives, 3
Quantifiable risk reduction, 117
Quantitative analytical results, 155
Quantitative nature, 106
Quantitative performance
  attributes sampling plans, 93
Quantitative risk assessments, 33–35, 39, 42–45, 48

R
Ready-to-eat (RTE) deli-meats, 357
  risk factors in (*see Listeria monocytogenes*)
Ready-to-eat foods, 168, 266
Reasonableness, 50
Reduced sampling, 251–254
  minced meat, 257
  skip-lot sampling, 260–262
Refrigerated storage, 15
Regulatory authorities, 200
  MC, 120, 121
Reiter's syndrome, 437
Relaying process, 420
Removing pathogens, 64
Representative sample, 140
Resident microorganisms, 269
Retention samples, 202
Risk analysis (RA), 1, 10
Risk assessment, 22
Risk characterization, 54, 55
Risk estimate, 52
Risk management
  concept, 4
  measures, 38
  metrics, 1
  principles, 67
Risk managers, 6, 7, 31
Risk profile, 7
Risk-based metrics
  ALOP, 3
  CAC, 5
  Codex system, 3
  framework, 3
  FSO (*see* Food safety objectives (FSOs))
  HACCP-based system, 3
  ICMSF, 4
  risk analysis, 5
  risk management, 4
  stakeholders, 5
  WTO/SPS agreement, 3

Risk-based public policy, 38
Rotavirus, 15
Rough risk assessment, 7
Routine testing program, 281

S
Safety assessment, 55
Salmonella, 19, 205, 209, 270
  milk powder, 243
  S. enterica, 210, 268
  sampling plans, 242, 244
  Schwarzengrund, 268
  Typhimurium, 268
Salmonella enterica, 222
Salmonellae, 13, 181
Salmonellosis, 20, 77
Sample handling, 199
Sample units
  collection
    food chain, 198
    investigative samples, 199
  containers, 199
  dry foods, 197
Sampling
  analytical procedure, 142
  collection, 198, 200
  confidence, 141–142
  equipment, 199–200
  food commodities, 201
  group of units, 132
  handling procedures, 142
  hazard, 142
  implement, 132
  label, 202
  liquids, 202
  lot, 140
  materials, 280
  microbiological, 92, 93
  OC function (*see* Operating characteristic (OC) function)
  packaging material, 200
  population, 132–133
  practical limitations, 143
  record of consistency, 143
  representative, 140–141
  sample units, 132–133
  stratification, 143
  uniformly, 143
Sampling plans
  acceptance/rejection, 140
  application, 184
  arithmetic means, 190, 194
  attributes, 93
  choice of, 142
  2-class and 3-class, 182
  decision-making criteria, 133
  definition, 133
  design, 142
  discriminating, 133
  enrichment procedures, 224
    E. coli O157:H7, 223, 224
    LOD, 221
    target microorganism, 222–225, 227, 228
  food class, 180
  geometric means, 190, 191
  hazard, 166, 189
  health hazard, 179, 186
  ICMSF, 189, 195
  microbial injury
    E. coli, 220, 221
    S. enterica, 221, 222
    stress, 220
  OC (*see* Operating characteristic (OC) function)
  OC curve, 142
  performance calculation, 189
  presence/absence testing, 133
  sampling scheme, 142
  selection process, 250
  statistically-based, 131
  three-class, 161
  two-class *vs*. three-class, 157, 159
  types, 12
  utility, 186
  value of *m* and *M*, 183, 184
Sampling procedures, 126
Sampling scheme, 142
Sampling tools, 280
Sanitary and Phytosanitary (SPS) agreement of WTO, 1
Scombroid (histamine) fish poisoning, 16
Seafood toxins, 16
Second party audits, 114
Serving size, 134
Shelf-life, 19
Shelf-life limits, 339, 376, 398
Shelf-life sampling, 299
Shelling plants, 341
Shiga toxin-producing *E. coli* (STEC), 14, 271
Shiga toxins (STs), 14
Shigella spp., 14
Shigellae, 78, 79
Shigellosis, 77
Skip-lot sampling
  acceptance probabilities, 253
  ANSI/ASQC, 251
  dairy product, 261
  implementation, 252–254
Small Round Structured Viruses (SRSVs), 15
Specificity and sensitivity, 236
Spore-formers, 272
SPS agreement, 106
Standards
  acceptance criteria, 107
Staphylococcus aureus, 181, 271, 283

Statistical process control (SPC)
auto-correlation, 310–311
control chart methodology, 303
control charts, 303, 314 (*see also* Control charts)
control criteria, 295
control factors, 295
controlled processes, 296
controlling product quality, 296
CPs and CCPs, 294
cumulative sum (Cusum) charts, 305
data organizing and interpretation, 295
degree of Variability (*see* Degree of variability)
food operations, 293
HACCP systems, 293, 294
hypothetical examples, 305, 306
influence variability, 295
informatics system, 296
internal temperature during cooking, 303
lot acceptance sampling, 294
microbial counts, 312, 313
microbial population density, 311
microbiological testing, 293, 304
monitoring procedures, 295
MR charts, 314
multiple charting, data subsets, 323
one-sided process charts, 305
performance criterion, 304
periodic testing, 305
presence/absence determinations, 315, 316 (*see also* Process capability study)
process control testing, 324, 325
process flow diagrams and mapping techniques, 294
process-oriented control systems, 294
significant hazards, 295
"stable appearing" process, 314
Statistical process control (SPC) (*cont.*)
tools, 296
unrecognized factors/unforeseen events, 296
variable control chart construction, 307–310
variables charts, quantitative data, 307
Statistical process control (SPC) industrial process control technology systems, 293
Status quo level, 35
Storage, 203
Storage temperature, 179
Strain variability, 363
Stratified random sampling approach, 133, 141
Stringency
FSOs/POs, 57
OC function, 138
Stringent sampling plans
conceivable alternative plans, 254, 255
erroneous procedures, 255
Supplier acceptance
auditing food operations, 113–115
Supplier audits, 114
System thinking, 9

T
The Management of Pathogens in Foods in International Trade, 3
The Principles for the Establishment and Application of Microbiological Criteria for Foods, 3
Thermal process, 374, 375
Third party audits, 114
Third party standards, 110
Three-class attributes plans, 147–152
Thrombotic thrombocytopenic purpura, 388
Tightened sampling
aseptically filled products, 258
attribute/variables sampling, 240
biased sampling, 241
control authorities, 243
food product, 239, 240
GHP and HACCP, 245
NFDM, 249
PCR, 244
preventing recurrence, 239
random sampling, 241
Salmonella, 242, 244
toxic contaminants, 247
two class plans, 245–248
zero tolerance, 247, 250
Tolerable level of risk (TLR)
and ALOP, 34, 35
and benefits, 38
consumer protection, 38
cost-benefit analysis, 37
established, 34
food control requirements, 37
food safety management, 36
foodborne diseases, 36
hazards, 36
public health, 37
reductions, 37
risk management measures, 38
societal matter, 34
sound scientific principles, 34
Toxigenic fungi, 17
Toxins/metabolites, 123
Transient microorganisms, 269
Transportation, 203
Trichinellae, 77
Trichinosis, 70, 76, 77
True probability, 132
Two-class attributes plans, 145–148
Two-class *vs*. three-class sampling plans, 157–159
Typhoid fever, 77, 78

U
US Department of Agriculture (USDA) monitoring program, 361
USDA Pathogen Modeling Program, 225
USDA-FSIS, 79

V
Vaccination control, 423
Validation, 213
Value of *m* and *M*, 183, 184
Variable control chart construction, 307–310
Variables plans
  decisions, 153
  identification, 153
  operation
    GMP, 154, 155
    k_1, selection of, 154
    limit value *V*, 154
  vs. three-class attributes, 162, 163
Verocytotoxin-producing *Escherichia coli* (VTEC), 271
Verocytotoxins (VTs), 14
Viruses, 15

W
Warm ambient (non-refrigeration) temperatures, 13
Wet-mix process, 345
Whole genome sequencing (WGS) techniques, 33
World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (WTO/SPS agreement), 3, 4

Y
Yersinia enterocolitica, 14
Yersinia pseudotuberculosis, 14, 272

Z
Zearalenone, 17
Zero cases, 56
Zero-risk, 20, 21
“Zero tolerance” policy, 19, 369
Zone concept, 277, 278